Supporting information

High Rates and Substrate Selectivities in Water by Polyvinylimidazoles as Transaminase Enzyme Mimics with Hydrophobically Bound Pyridoxamine Derivatives as Coenzyme Mimics

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Contents

1. General information.

Chemicals: Solvents, inorganic salts, and organic reagents were purchased from commercial resources and used without further purification unless otherwise mentioned. A commercial initiators azobis(isobutyronitrile) (AIBN) was purified by crystallization from chloroform/methanol.

Chromatography: Merck pre-coated 0.25 mm silica plates containing a 254 nm fluorescence indicator were used for analytical thin-layer chromatography. Flash chromatography was performed on 230-400 mesh silica (Silica Gel 60) from Silicycle.

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.

Spectroscopy: NMR spectra were obtained on a Bruker DPX 300 or 400 MHz spectrometer. UV-vis spectra were taken on a Varian Cary IE UV-vis spectrometer. CI MS spectra were taken on a Nermag R-10-10 instrument. FAB MS spectra were taken on a JEOL JMS-DX-303 HF instrument using either glycerol or p-nitrobenzyl alcohol as matrices. Matrix assisted laser desorption ionization (MALDI) mass spectra were acquired using an Applied Biosystems Voyager DE Pro time-of-flight mass spectrometer. Positive ion mass spectra were acquired in the linear mode using a nitrogen laser (337nm). Instrument settings were as follows: accelerating voltage, 21,000 volts; grid voltage, 95%; guide wire, 0.05%; extraction delay time, 200 nsec. All data processing was performed using Applied Biosystems Data Explorer v 4.0.0.0.

2. Syntheses.

2.1 Synthesis of 4-vinylimidazole by decarboxylation of urocanic acid

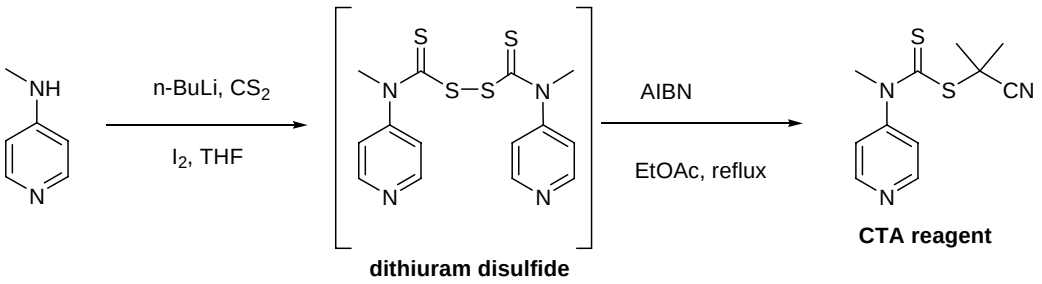
Anhydrous urocanic acid (5.0 g, 36.29 mmole) was heated *in vacuo* in a distilling apparatus. The product distilled, as a colorless viscous liquid which crystallized in the receiver, on careful heating at 220-240 °C; 1.7g (50%, 18.08 mmol). mp 82.0-84.0 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 11.95 (br s, 1H); 7.61 (s, 1H), 7.11 (s, 1H), 6.62 (dd, J = 17.6 Hz, 11.2 Hz, 1H), 5.66 (d, J = 17.6 Hz, 1H), 5.11 (d, J = 11.2 Hz, 1H);

^{13}C NMR (75 MHz, $CDCl_3$) 112.0, 119.6, 126.3, 135.3, 135.6. This is a known compound and the spectral data are consistent with those reported in literature.[1]

2.2 Synthesis of 1-dodecyl-4-vinylimidazole

4-Vinylimidazole (470 mg, 5.0 mmole) was dissolved in 1mL of dry THF and added drop wise to a solution of NaH (60%) (200 mg, 5.0 mmole) in 1 ml of dry THF at 0 ^oC. The mixture was stirred at 0 ^oC for 15 minutes before the addition of 1-iodododecane (1.23 ml, 5.0 mmole) in 2 ml of dry THF. The reaction mixture was stirred for an additional 2h at room temperature then the solvent was removed by rotavap. The crude reaction mixture was purified by column chromatography (hexane : EtOAc =50:1) to provide the desired product (945 mg, 3.61 mmol, 72%). The NMR spectrum indicates that is 83% 4-vinyl-1-dodecylimidazole and 17% 5-vinylimidazole mixture. The major 4-vinyl-1-dodecylimidazole was isolated by preparative TLC plate (Hexane : DCM =70:30). ^1H NMR (300 MHz, $CDCl_3$) 7.40 (s, 1H), 6.86 (s, 1H), 6.59 (dd, J = 17.4 Hz, 10.8 Hz, 1H), 5.82 (d, J = 17.4 Hz, 1H), 5.11 (d, J = 10.8 Hz, 1H)), 3.87 (t, J = 6.9 Hz, 1H), 1.79-1.75 (m, 2H), 1.26-1.24 (m, 18H), 0.88 (t, J = 6.0 Hz, 3H); ^{13}C NMR (100 MHz, $CDCl_3$) 137.1, 128.7, 116.4, 111.5, 46.9, 31.8, 30.9, 30.8, 29.5, 29.4, 29.3, 29.2, 28.9, 26.4, 22.6, 14.0; CI MS: 263.17 (M+1), 525.23 (2M+1).

2.3 Synthesis of the 2-cyanopropan-2-yl N-methyl, N-(pyridine-4yl) carbamodi-thioate (CTA reagent)



To a solution of 4-(methylamino)pyridine (1.00g; 9.25 mmol) in dry THF (60mL) at -10 ºC, was added *n*-butyl lithium (1.6M in hexane) (6.5 mL) over 10 minutes under an inert atmosphere. The resultant pale yellow mixture was allowed to stir at -10 ^oC for an hour and then warmed to 0ºC before the dropwise addition of carbon disulfide (0.9 mL) and the yellow suspension was allowed to stir at room temperature for a

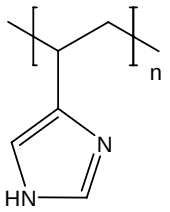
[1] C. A. Faler and M. M. Joullie *Org. Lett.* **2007**, *9*, 1987.

further one hour. The resultant mixture was cooled to 0 ^{o}C and oxidized by the addition of an iodine solution (1.23 g iodine dissolved in 25 mL of 10% KI aqueous solution) to yield the intermediate dithiuram disulfide as yellow solid (1.28 g, 76% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 8.78 (dd, J = 6.0 Hz, 1.5 Hz, 2H); 7.43 (dd, J = 6.0 Hz, 1.5 Hz, 2H); 3.80 (s, 6H). This is a known compound and the spectral data are consistent with those reported in the literature.[2]

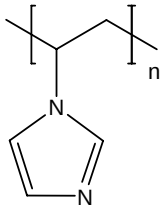
2-Cyanopropan-2-yl *N*-methyl, *N*-(pyridin-4-yl)carbamodithioate (CTA reagent). A solution of 2,2'-azobisisobutyronitrile (206 mg, 1.26 mmol) and the intermediate dithiuram disulfide (230 mg, 0.628 mmol) in ethyl acetate (10 mL) was heated at reflux for 16 h. After removal of the volatiles *in vacuo*, the crude product was subjected to column chromatography with *n*-hexane:ethyl acetate (v:v) as eluant to afford the desired 2-cyanopropan-2-yl-*N*-methyl-*N*-(pyridin-4-yl)carbamodithioate (**CTA reagent**) as 278 mg, 88 % yield,which solidified when stored in a freezer (-15 ^{0}C). ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.83 (s, 6H); 3.69 (s, 3H); 7.20 (d, J = 4.5 Hz, 2H); 8.76 (d, J = 4.5 Hz, 2H). This is a known compound and the spectral data are consistent with those reported in literature.[2]

Polymerizations

2.4 Synthesis of poly-4-vinylimidazole and 1-vinylimidazole



P4VIm



PNVIm

A typical experimental procedure follows: the monomer, AIBN, CTA reagent and 1 mL of distilled benzene or a mixture of distilled chlorobenzene : acetonitrile (v/v) were mixed in a sealed tube, degassed by three repeated freeze-evacuate-thaw cycles and sealed. The mixture was heated at 80°C for 36-48 h under nitrogen. The reaction mixture was cooled to room temperature, washed with toluene and collected as a

[2] M. Benaglia, J. Chiefari, Y. K. Chong, G. Moad, E. Rizzardo, and S. H. Thang *J. Am. Chem. Soc.* **2009**, *131*, 6914.

white solid which was dried at 120 ^{o}C under reduced pressure for 24 h to give the desired polymers.

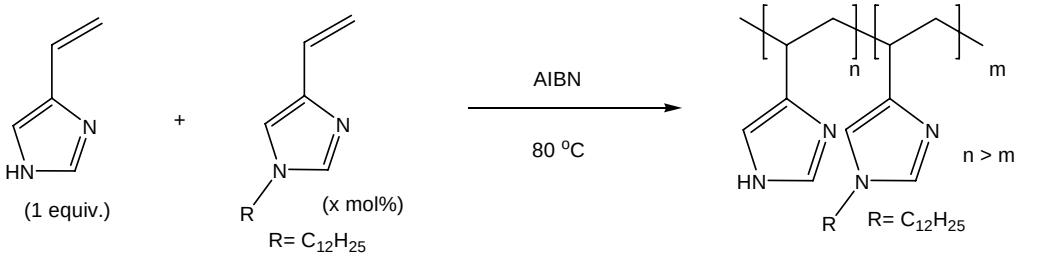
Poly-4-vinylimidazole: Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (75 mg). ^{1}H NMR (400 MHz, DMSOd$_6$ / TFAd$_1$) 8.82-8.40 (br, 1H), 7.20-6.80 (br, 1H), 2.10-1.45 (br, 3H).

Poly-1-vinylimidazole: Following the above general procedure with 1-vinylimidazole (100 mg, 1.064 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (89 mg).^{1}H NMR (400 MHz, DMSOd$_6$ / TFAd$_1$) 7.50-6.60 (br, 3H), 3.26-2.70 (br, 1H), 2.30-1.60 (br, 2H),

Poly-4-vinylimidazole (Table 1, entry 9): Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), AIBN (5 mg, 0.05319 mmol) in a mixture of chlorobenzene : acetonitrile (v/v), the desired polymer was obtained as a white solid (85 mg). ^{1}H NMR (400 MHz, CD$_3$OD) 7.21-7.73 (br, 1H), 6.14-6.65 (br, 1H), 1.30-2.40 (br, 3H).

Poly-4-vinylimidazole (RAFT modification method (Table 1, entry 10)): Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), AIBN (5 mg, 0.053 mmol) and 2-cyanopropan-2-yl-N-methyl-N-(pyridine-4-yl)carbamodithioate (22.19 mg, 0.088 mmol)as chain transfer agent (CTA) in a mixture of chlorobenzene : acetonitrile (v/v), the desired polymer was obtained as yellowish solid (85 mg). ^{1}H NMR (400 MHz, CD$_3$OD) 7.98 (d, J = 6.0 Hz, 1H, CTA reagent), 7.67-7.56 (br, 1H, imidazole), 6.56 (d, J = 6.0 Hz, 1H, CTA reagent), 6.31-6.0 (br, 1H, imidazole), 2.83 (s, 3H), 2.38-1.30 (br, 3H (imidazole) + 6H (CTA reagent)).

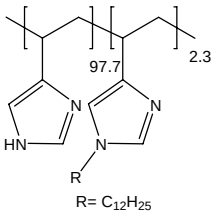
2.5 Synthesis of copolymers



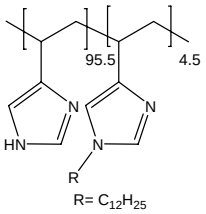
4VIm **1-dodecyl-4-vinylimidazole** **partially dodecylated P4VIm**

A typical experimental procedure follows: the monomers (4-vinylimidazole (1 equiv.) and 1-dodecyl-4-vinylimidazole (x mol%), AIBN, and 1 mL of distilled

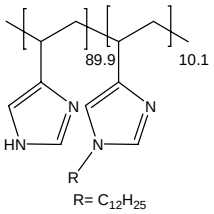
benzene or a mixture of distilled chlorobenzene : acetonitrile (v/v) were mixed in a sealed tube, degassed by three repeated freeze-evacuate-thaw cycles and sealed. The mixture was heated at 80°C for 36-48 h under nitrogen. The reaction mixture was cooled to room temperature, washed with toluene and collected as a white solid which was dried at 120 ^{o}C under reduced pressure for 24 h to give the desired polymers. The dodecylation percentages were determined by ^{1}H NMR.



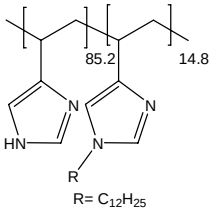
2.3% dodecylated poly-4-vinylimidazole: Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), 1-dodecyl-4-vinylimidazole (5.6 mg, 0.024 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (90 mg). ^{1}H NMR (400 MHz, $DMSOd_6$ / $TFAd_1$) 8.85-8.55 (br, 1H, imidazole), 7.25-6.85 (br, 1H, imidazole), 2.20-1.55 (br, 3H, (imidazole) + 2H (dodecyl group)); 1.4-0.8 (br, 23H (dodecyl group)). The ^{1}H NMR indicated a poly-4-vinylimidazole with 2.3% of dodecylation.



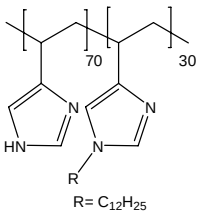
4.5% dodecylated poly-4-vinylimidazole: Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), 1-dodecyl-4-vinylimidazole (12.0 mg, 0.052 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (87 mg). ^{1}H NMR (400 MHz, $DMSOd_6$ / $TFAd_1$) 8.90-8.60 (br, 1H, imidazole), 7.30-6.80 (br, 1H, imidazole), 2.20-1.40 (br, 3H, (imidazole) + 2H (dodecyl group)); 1.35-0.75 (br, 23H (dodecyl group)). The ^{1}H NMR indicated a poly-4-vinylimidazole with 4.5% of dodecylation.



10.1% dodecylated poly-4-vinylimidazole: Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), 1-dodecyl-4-vinylimidazole (24.0 mg, 0.092 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (87 mg). ^{1}H NMR (400 MHz, $DMSOd_6$ / $TFAd_1$) 8.95-8.60 (br, 1H, imidazole), 7.25-6.75 (br, 1H, imidazole), 2.20-1.35 (br, 3H, (imidazole) + 2H (dodecyl group)); 1.30-0.70 (br, 23H (dodecyl group)).

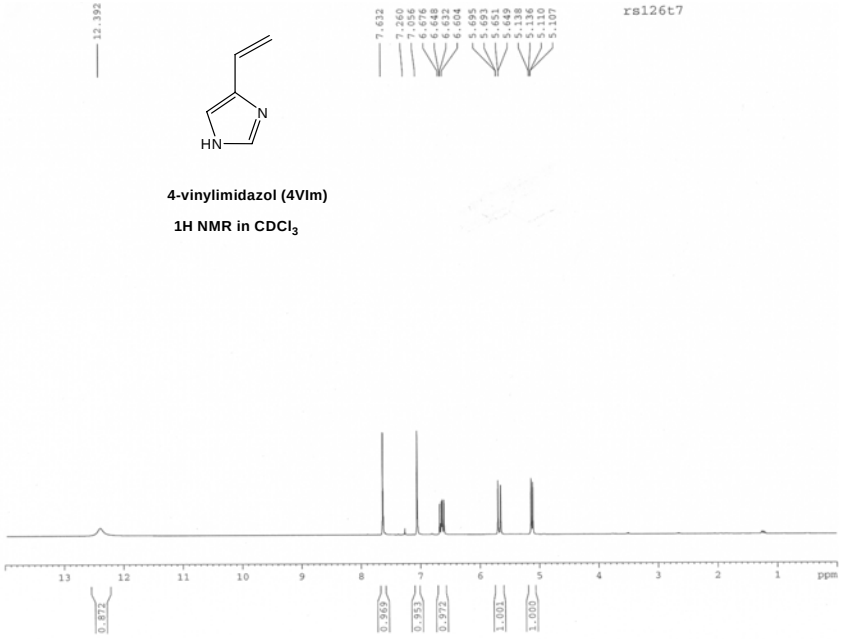


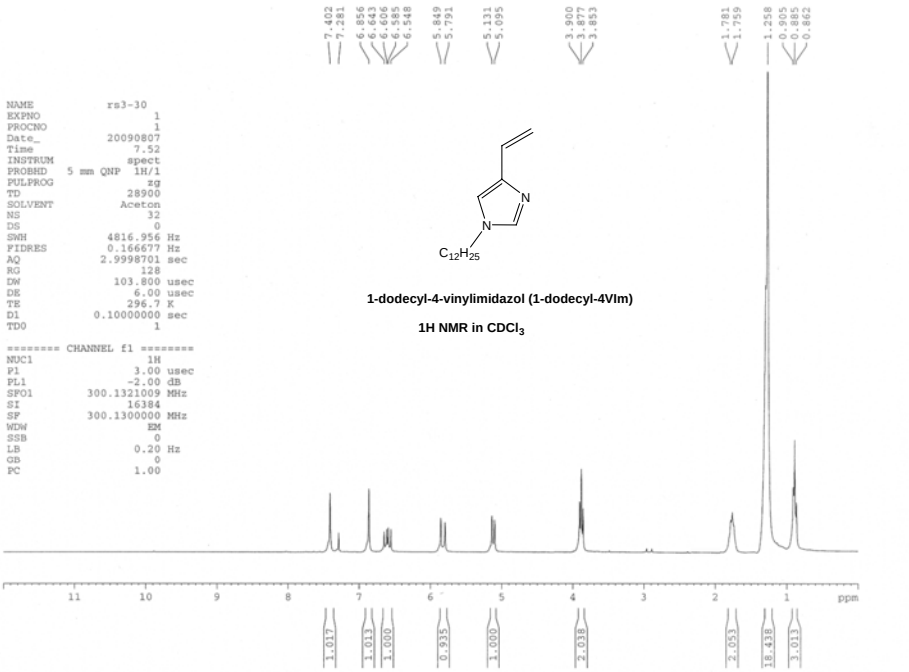
14.8% dodecylated poly-4-vinylimidazole: Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), 1-dodecyl-4-vinylimidazole (35.0 mg, 0.134 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (87 mg). ^{1}H NMR (400 MHz, $DMSOd_6$ / $TFAd_1$) 8.95-8.60 (br, 1H, imidazole), 7.30-6.70 (br, 1H, imidazole), 2.30-1.30 (br, 3H, (imidazole) + 2H (dodecyl group)); 1.30-0.70 (br, 23H (dodecyl group)). The ^{1}H NMR indicated a poly-4-vinylimidazole with 14.8% of dodecylation.



30% dodecylated poly-4-vinylimidazole: Following the above general procedure with 4-vinylimidazole (100 mg, 1.064 mmol), 1-dodecyl-4-vinylimidazole (66.9 mg, 0.255 mmol), AIBN (1.7 mg, 0.01064 mmol) in benzene, the desired polymer was obtained as a white solid (87 mg). ^{1}H NMR (400 MHz, $CDCl_3$ / $DMSOd_6$ / $TFAd_1$) 8.80-8.35 (br, 1H, imidazole), 7.30-6.80 (br, 1H, imidazole), 2.25-1.40 (br, 3H, (imidazole) + 2H (dodecyl group)); 1.30-0.80 (br, 23H (dodecyl group)). The ^{1}H NMR indicated a poly-4-vinylimidazole with 30 % of dodecylation.

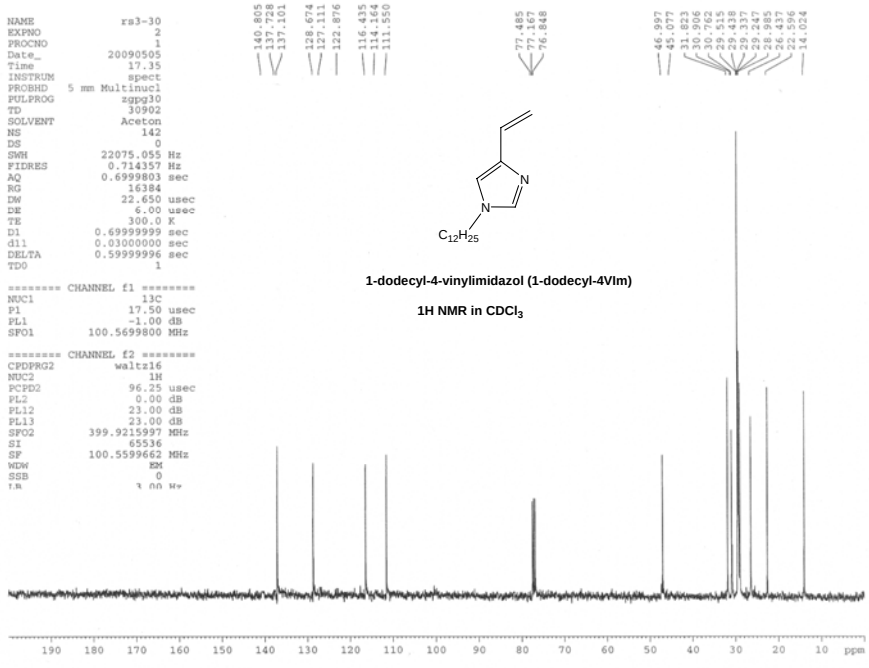
3. ^{1}H NMR spectra and MALDI-TOF data for all new monomers and polymers.





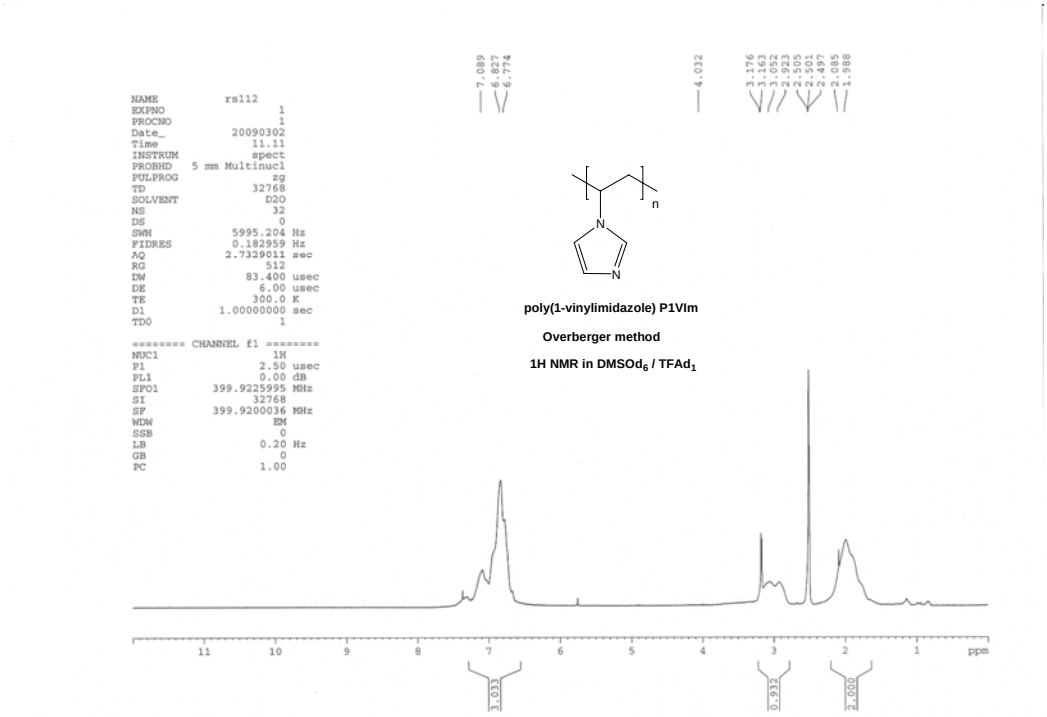
1-dodecyl-4-vinylimidazol (1-dodecyl-4VIm)

1H NMR in $CDCl_3$



1-dodecyl-4-vinylimidazol (1-dodecyl-4VIm)

1H NMR in $CDCl_3$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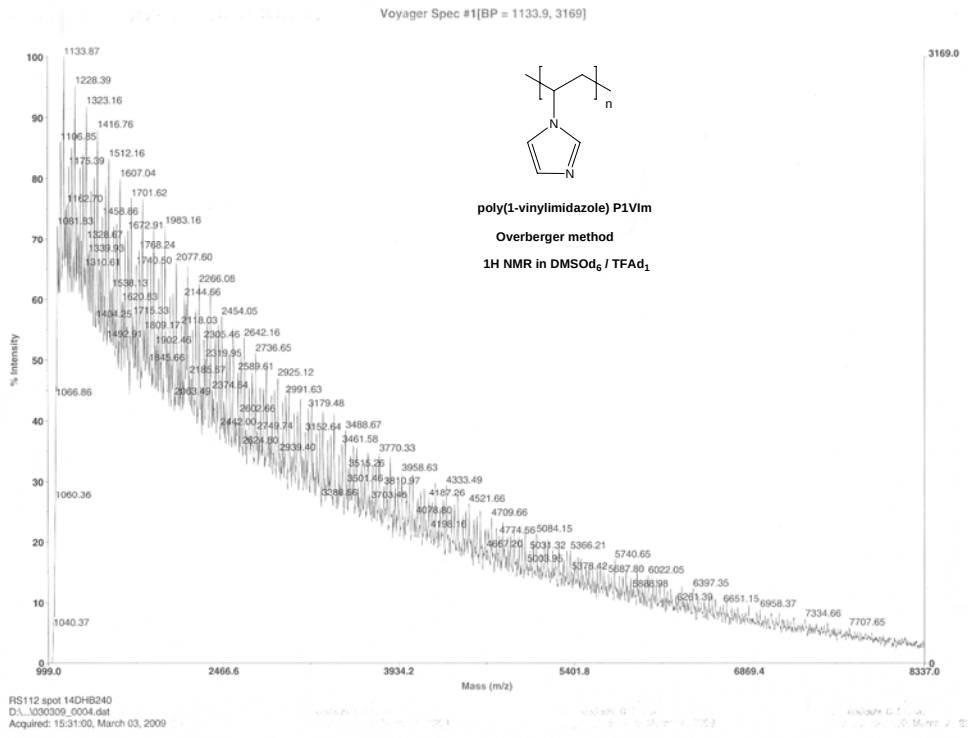
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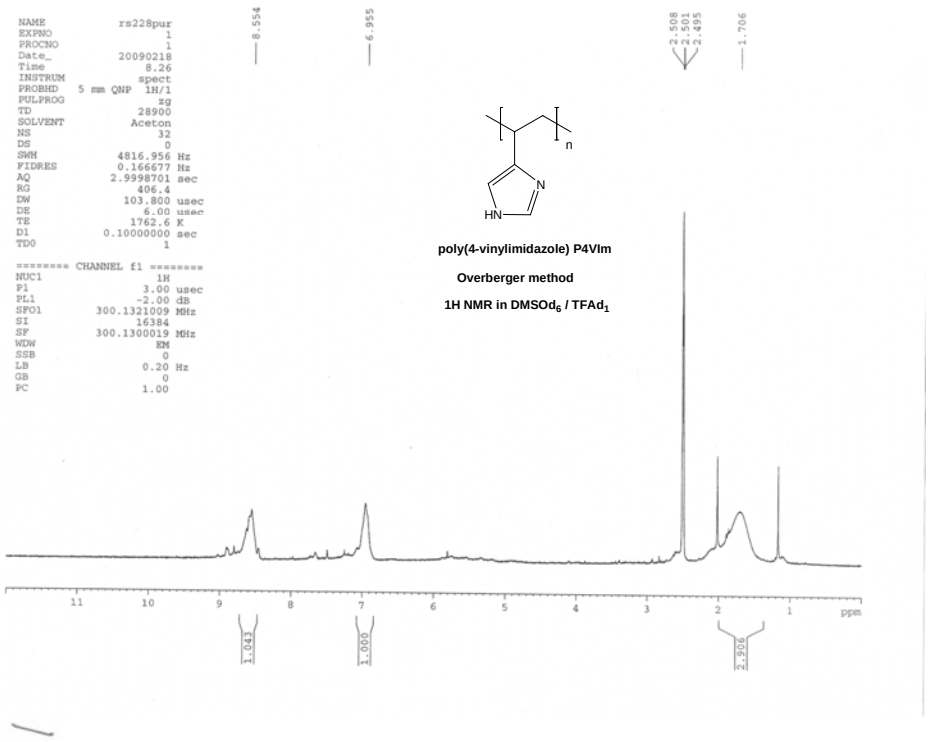
Overberger method
1H NMR in DMSOd6 / TFAd1



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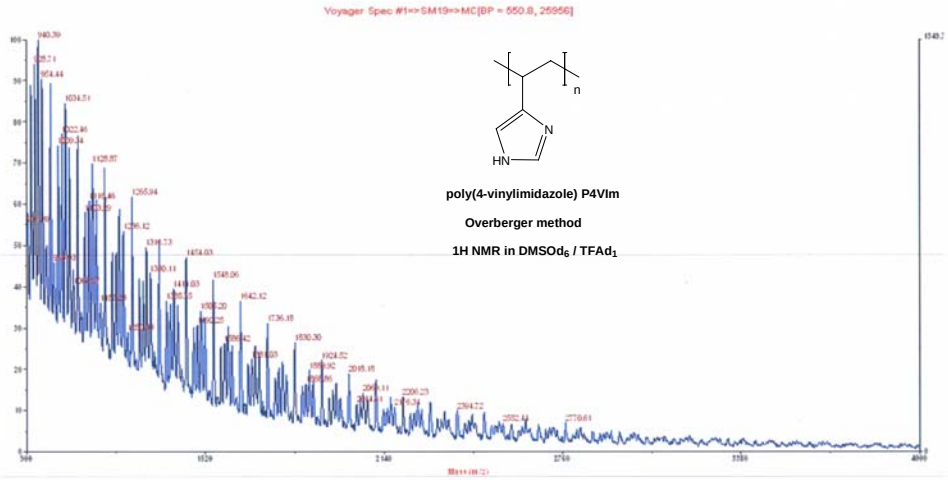
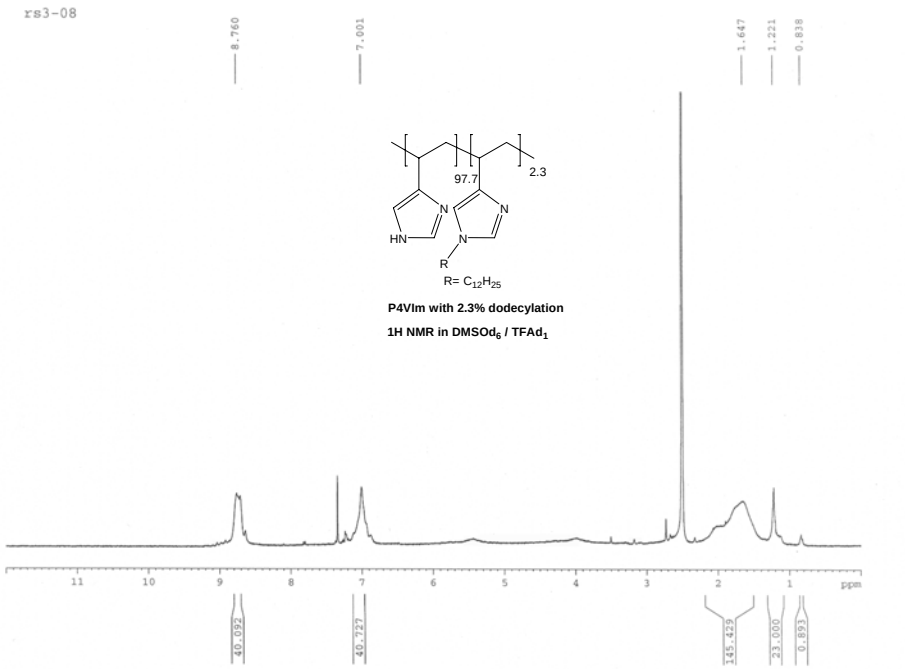
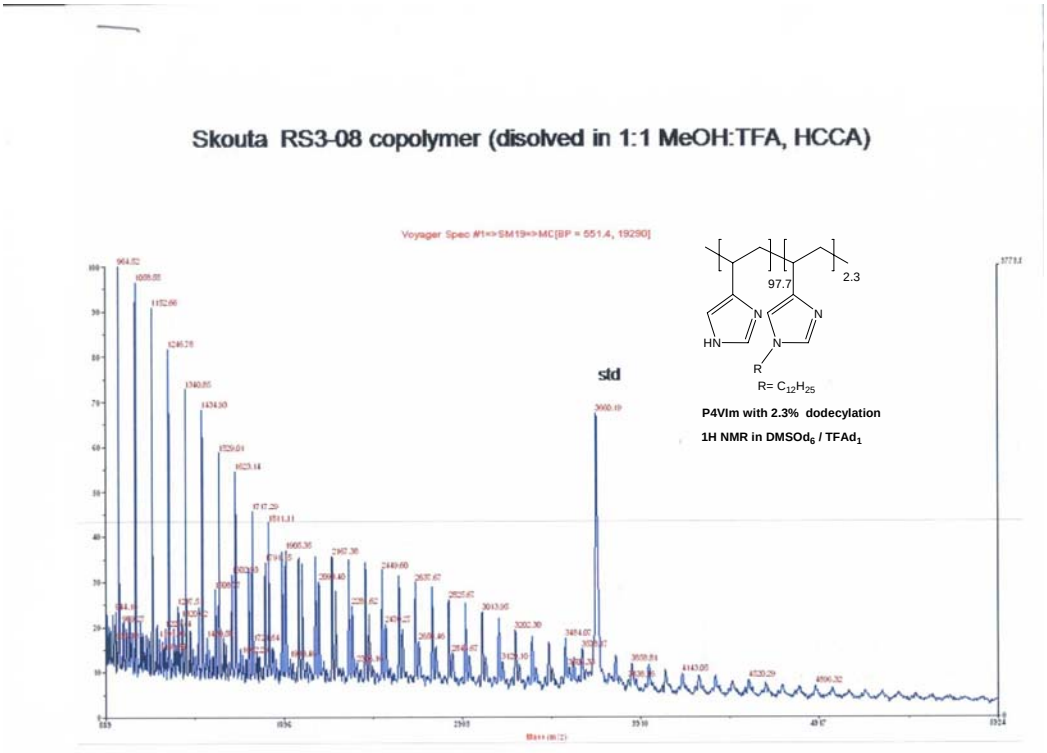




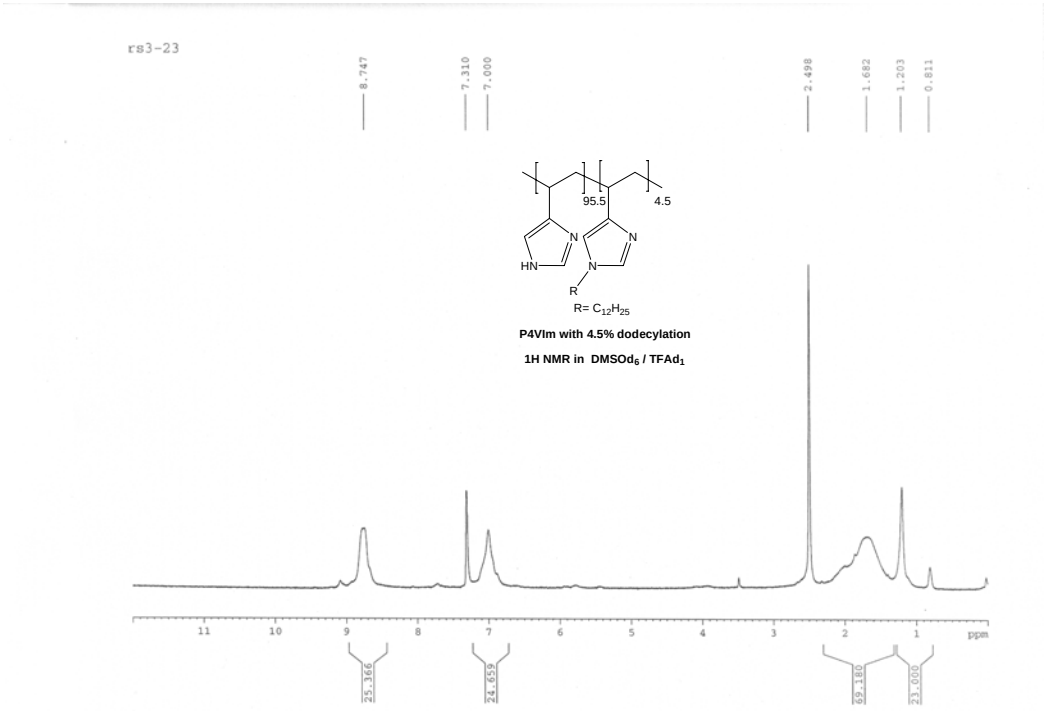
P4VIm with 2.3% dodecylation

1H NMR in $DMSOd_6$ / $TFAd_1$



P4VIm with 2.3% dodecylation

1H NMR in $DMSOd_6$ / $TFAd_1$



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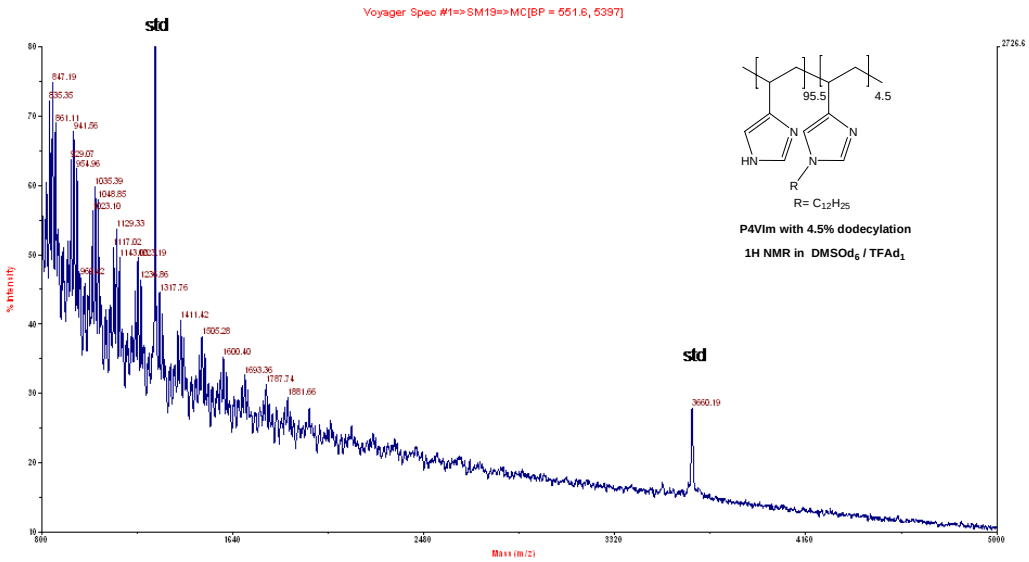

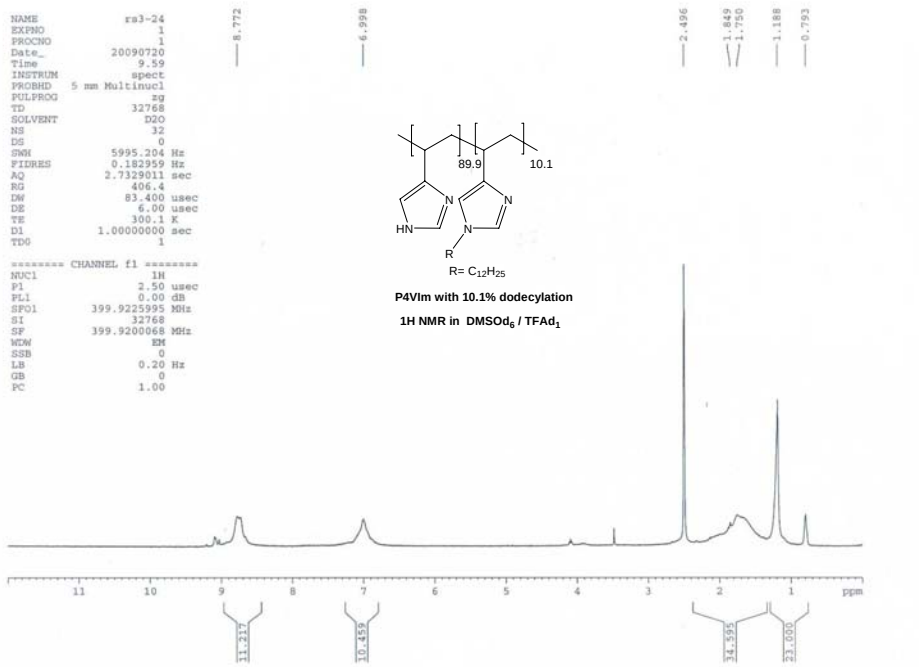

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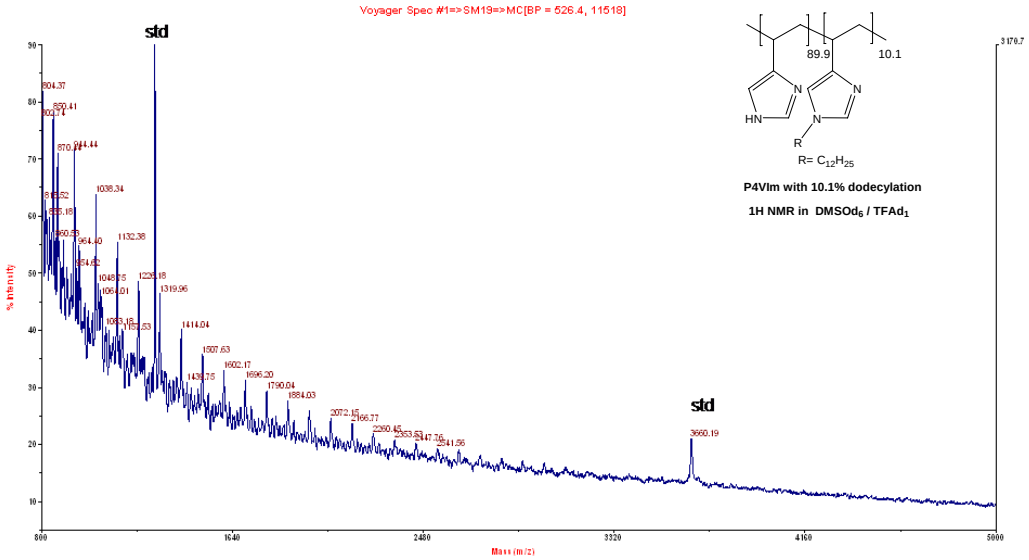

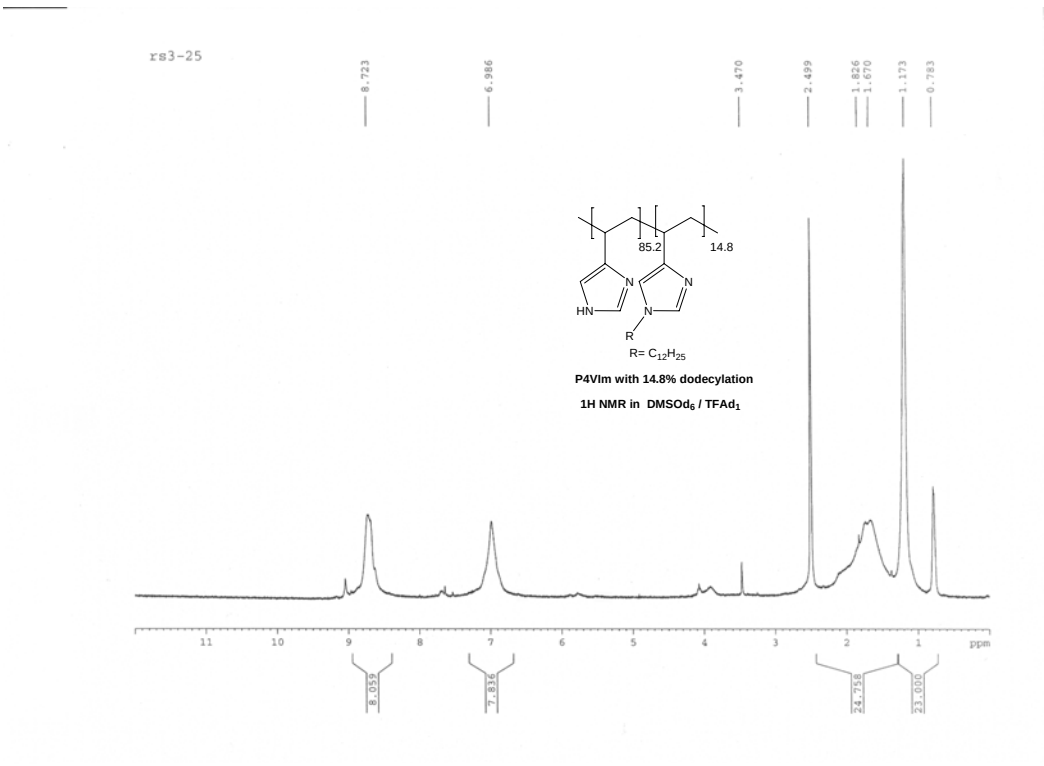

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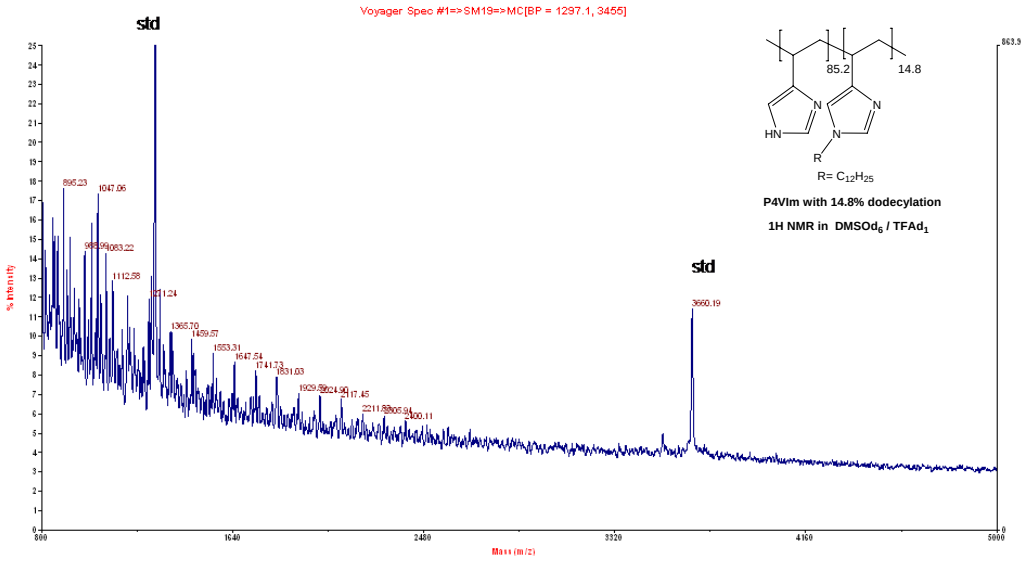
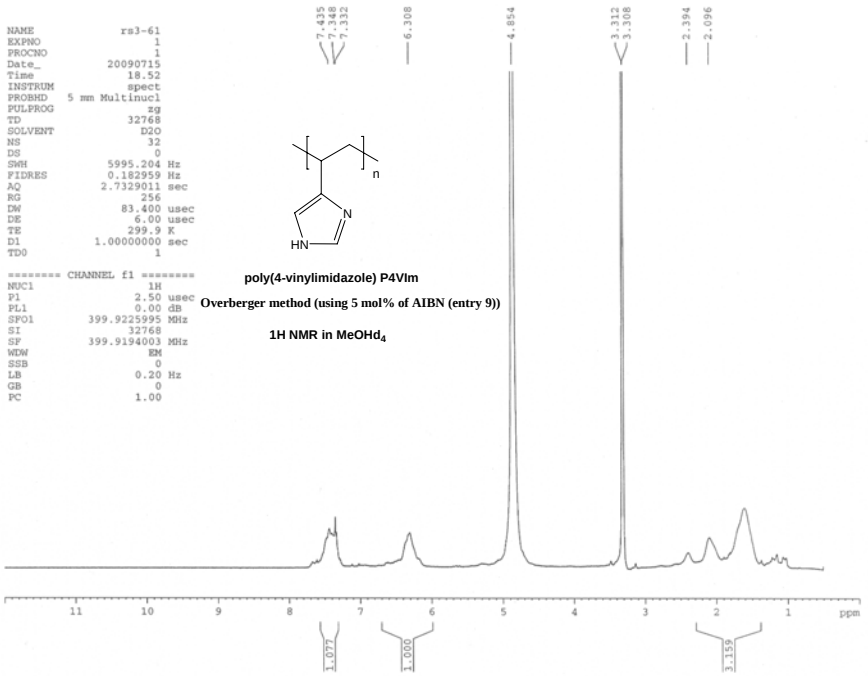
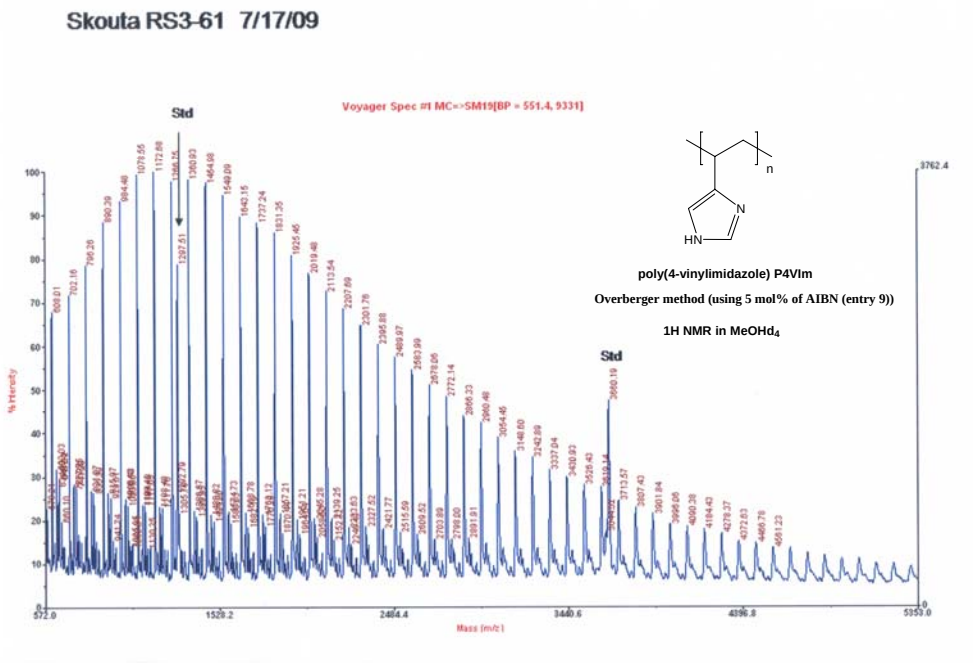




poly(4-vinylimidazole) P4VIm

Overberger method (using 5 mol% of AIBN (entry 9))

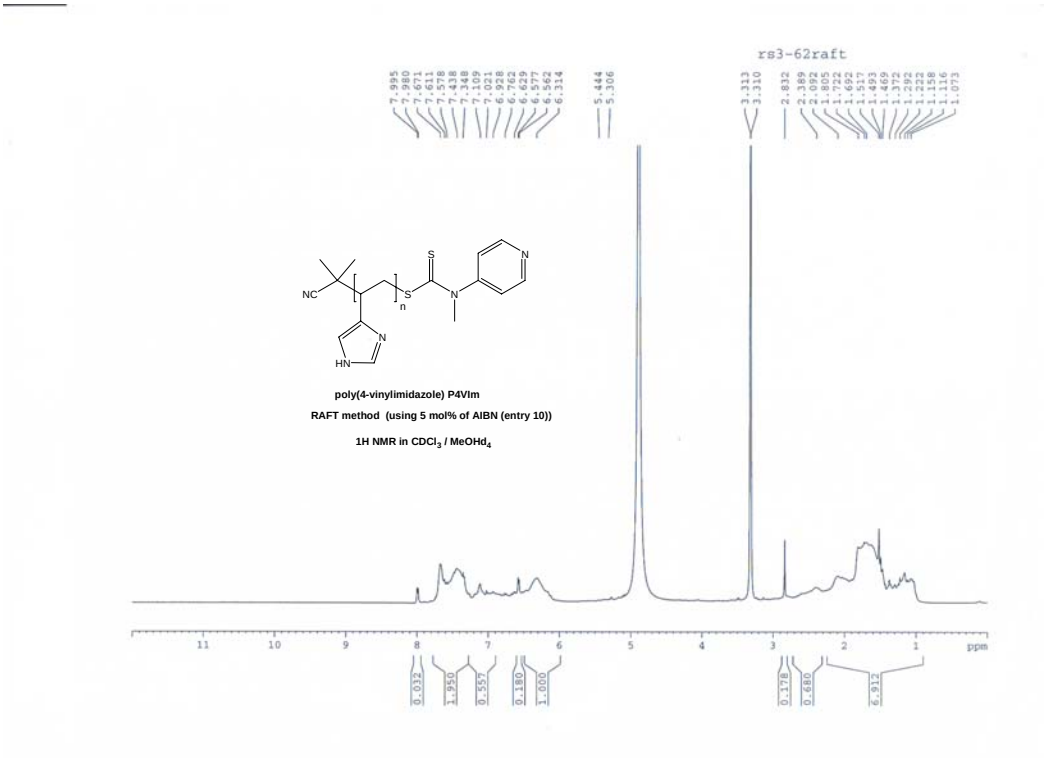
1H NMR in MeOHd$_4$

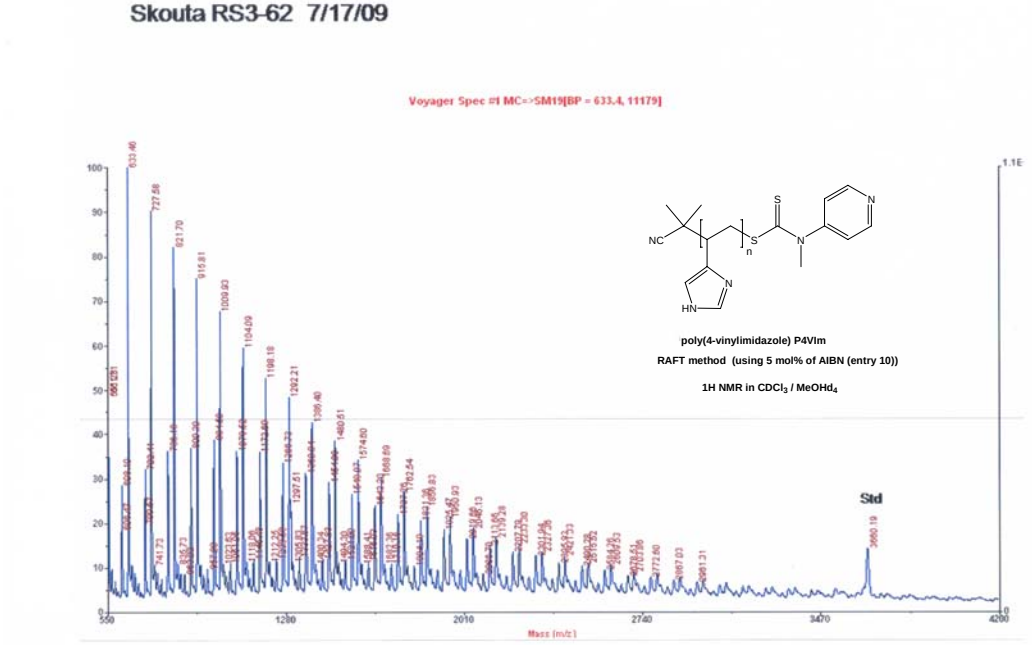


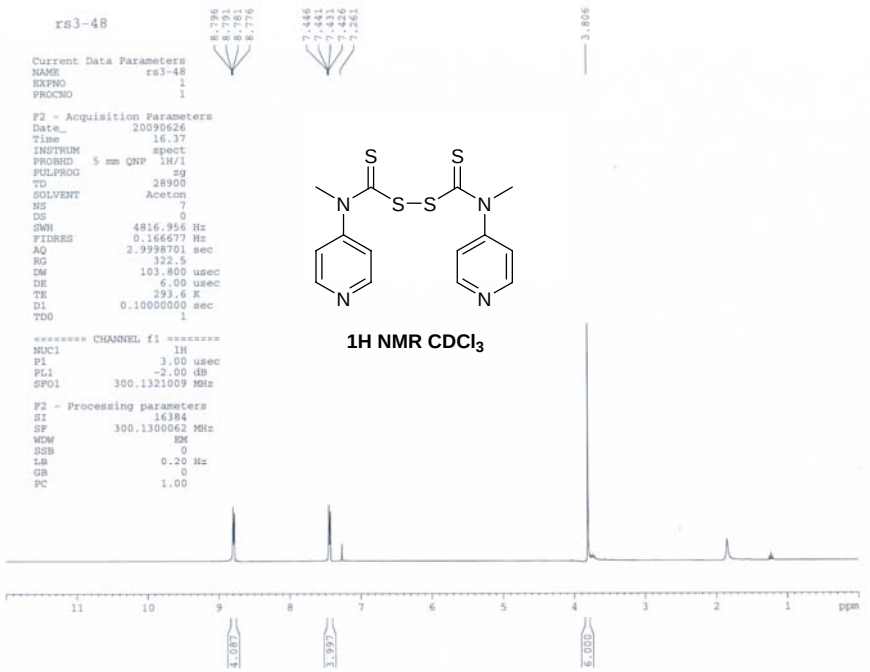
poly(4-vinylimidazole) P4VIm

Overberger method (using 5 mol% of AIBN (entry 9))

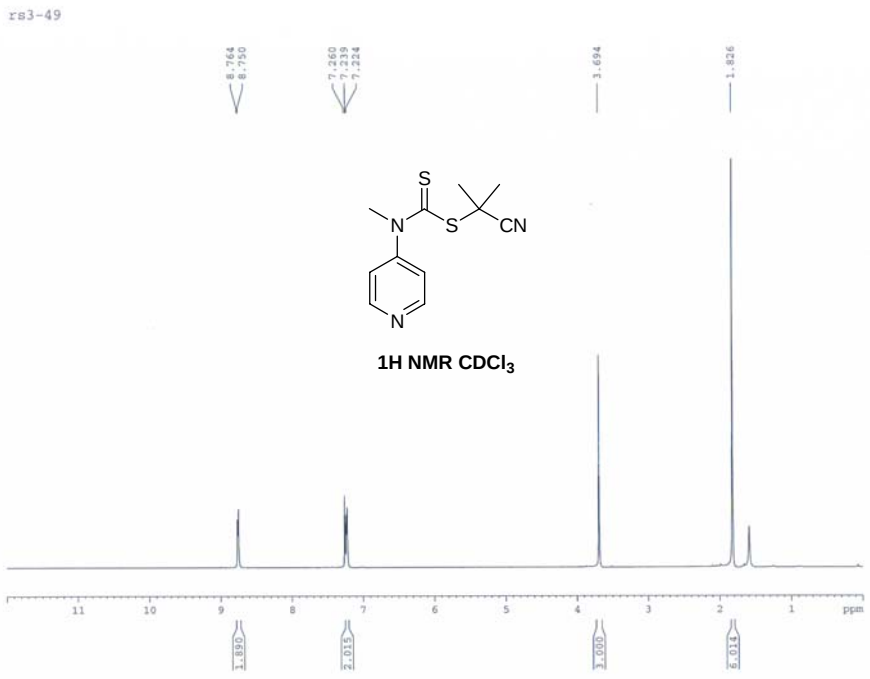
1H NMR in MeOHd$_4$


rs3-62raft
poly(4-vinylimidazole) P4VIm
RAFT method (using 5 mol% of AIBN (entry 10))
1H NMR in CDCl3 / MeOHd4
ppm


Skouta RS3-62 7/17/09
Voyager Spec #1 MC=>SM19[BP = 633.4, 11179]
poly(4-vinylimidazole) P4VIm
RAFT method (using 5 mol% of AIBN (entry 10))
1H NMR in CDCl3 / MeOHd4
Std
Mass (m/z)



1H NMR $CDCl_3$



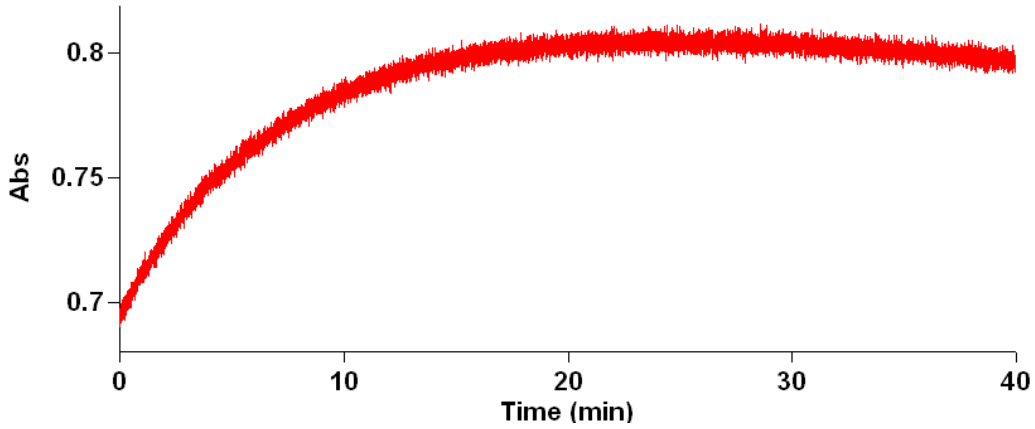
1H NMR $CDCl_3$

4. Kinetic study of transamination reactions using u.v.

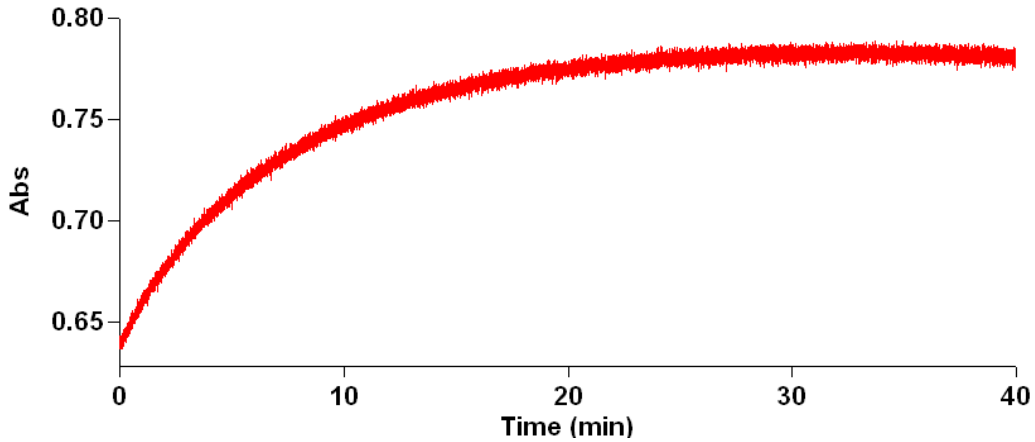
The following reagents was added in 1 mL u.v. cell,: pyridoxamine **E** (5 x 10^{-3} mol/L, 30 µL), P4VIm (0% dodecylated) or P4VIm (4.5% dodecylated) (1.5 g/L, 25 µL), pyruvic acid (5.0 x 10^{-2} mol/L, 100 µL) and ethylenediaminetetraacetic acid (EDTA) (2.0 x 10^{-3} mol/L, 845 µL, in N-[2-hydroxyethyl]piperazine N'-[2-ethanesulfonic acid] (HEPES) buffer (pH = 7.5). The rate of the reaction at pH = 7.5 and 20 ^{o}C was followed by the appearance of the u.v. spectrum of the product pyridoxal.

A representative u.v. spectrunm is provided here in the case of P4VIm with 2.3% dodecylation. A typical u.v. spectra of the increase of the product pyridoxal at 385 nm follows:

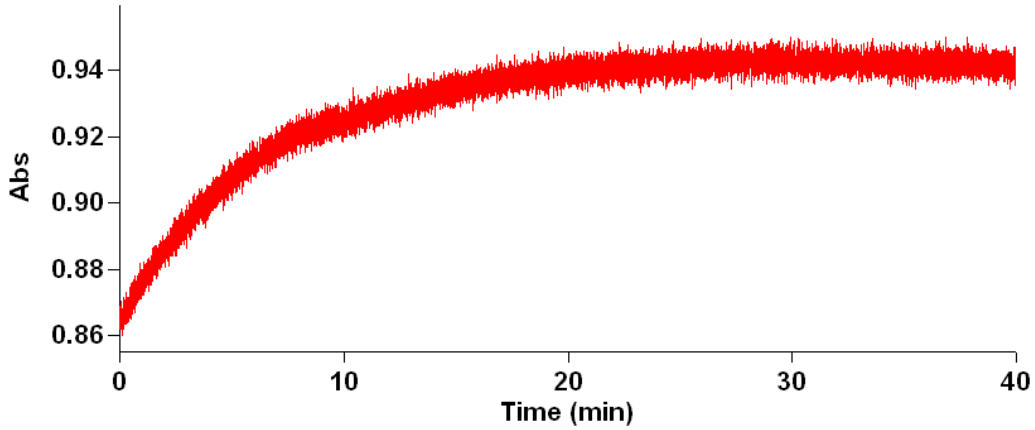
P4VIm



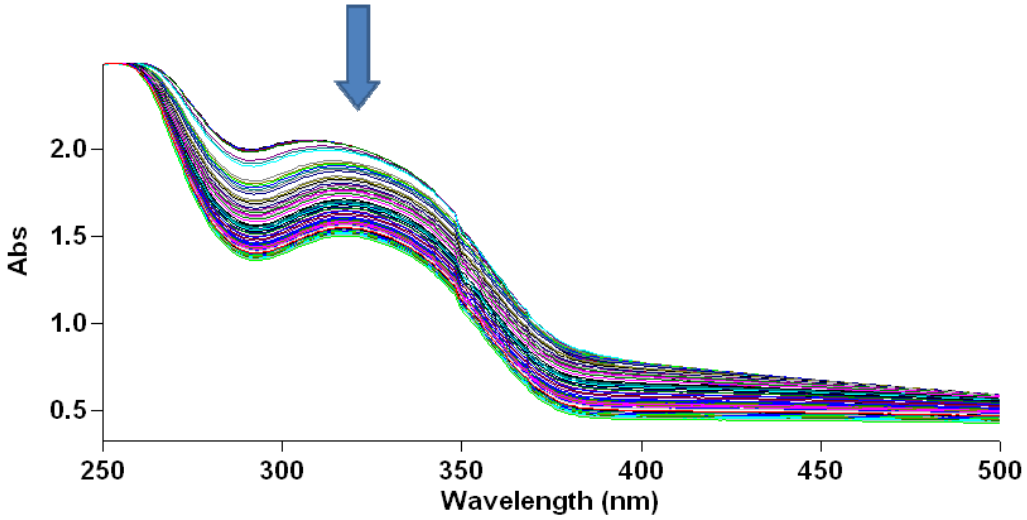
P4VIm with 2.3% dodecylation



P4VIm with 4.5% dodecylation

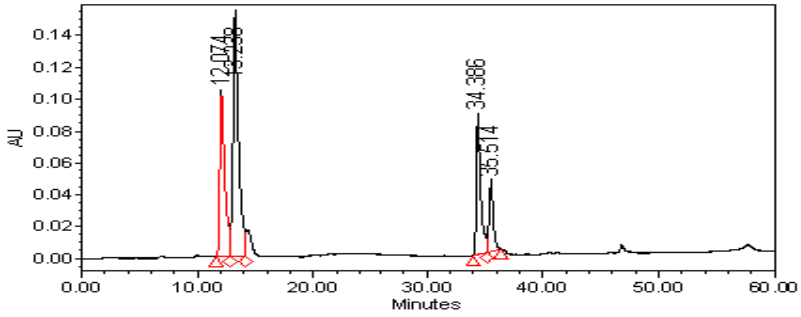


A typical decrease of in UV absorption at 325 nm (pyridoxamine) during the course of transamination.



5. Transamination and analysis of the amino acid products with HPLC.

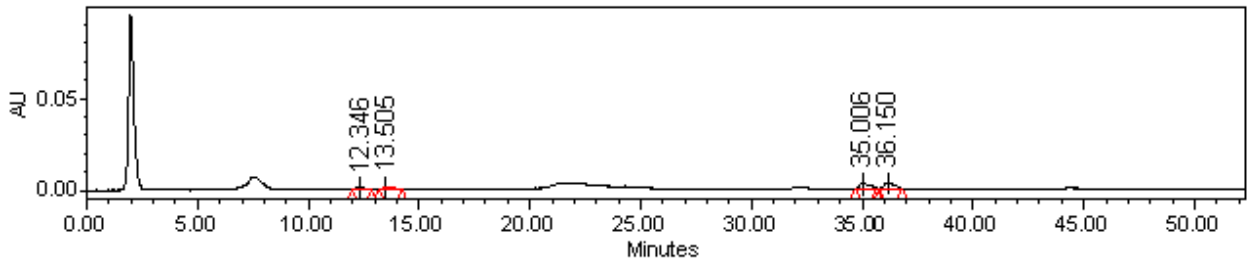
Methanolic solutions of pyridoxamine **E** (5 x 10^{-3} mol/L, 30 μL), P4VIm (0% dodecylated) or P4VIm (4.5% dodecylated) (1.5 g/L, 25 μL), pyruvic acid (5.0 x 10^{-2} mol/L, 100 μL), phenylpyruvic acid (5.0 x 10^{-2} mol/L, 100 μL), were mixed with EDTA (2.0 x 10^{-3} mol/L, 745 μL) in HEPES buffer (pH = 7.5) at room temperature. The reactions were run for 1h to 24 h. To a 200 μL of the mixture were added 10 μL of a derivatizing solution containing 0.2 M o-phthalaldehyde and 0.2 M *N*-Boc-cysteine/*N*-acetyl-cysteine in methanol, 10 μL of an aqueous buffer solution of 1.0 M K_2HPO_4 (pH 8.0). The resulting solutions were analyzed by HPLC, with UV-vis detection at 344 nm.[3] Standard samples were run before and after the samples to ensure the right retention times of the products. A representative HPLC trace is provided here.



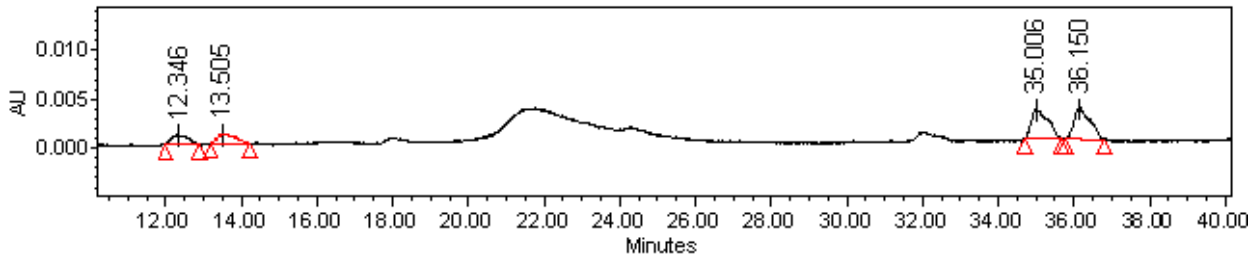
A representative standard sample: DL alanine / DL phenylalanine

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

Competition reaction between pyruvic acid and phenylpyruvic acid using P4Vim with 0% dodecylation



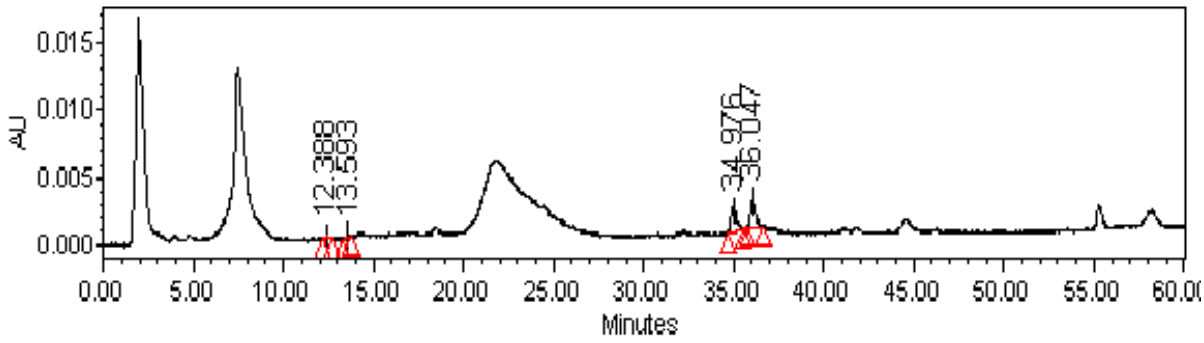
HPLC plot of the formation of D/L phenylalanine and D/L alanine



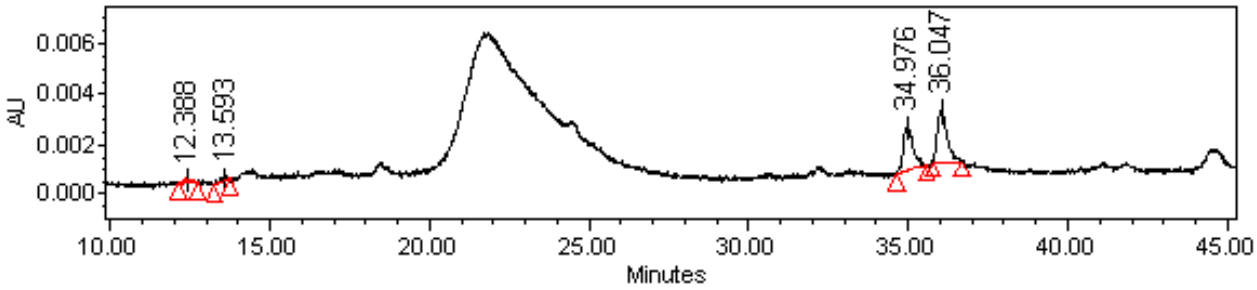
Zoom in of the HPLC plot

Name	Retention Time	Erea
1	12.346	28047
2	13.505	33967
3	35.006	89703
4	36.150	93927

Competition reaction between pyruvic acid and phenylpyruvic acid using P4Vim with 4.5% dodecylation



HPLC plot of the formation of D/L phenylalanine and D/L alanine

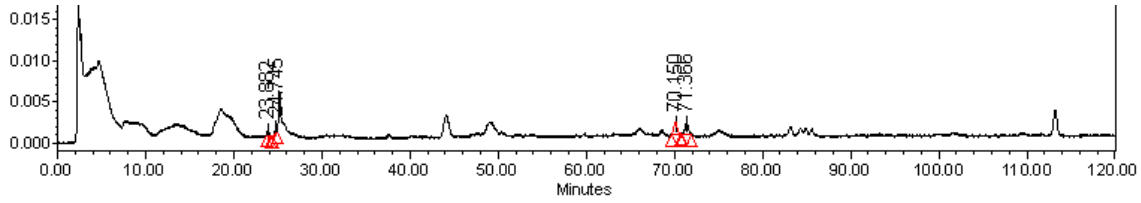


Zoom in of the HPLC plot

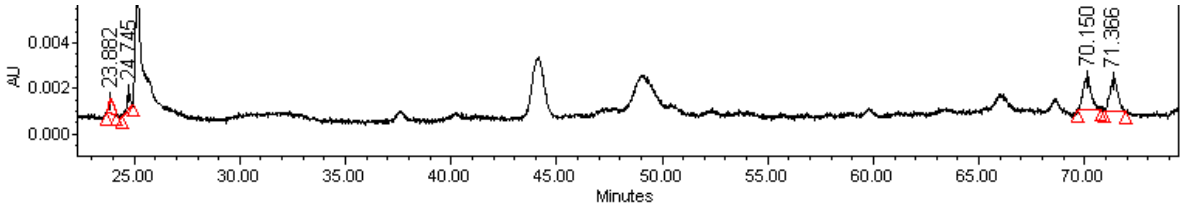
Name	Retention Time	Erea
1	12.388	2204
2	13.593	2165
3	34.976	40073
4	36.047	43027

Competition reaction between pyruvic acid and indole-3-pyruvic acid using P4Vim with 4.5% dodecylation

Methanolic solutions of pyridoxamine **E** (5 x 10^{-3} mol/L, 50 µL), P4VIm (4.5% dodecylated) (1.5 g/L, 25 µL), pyruvic acid (5.0 x 10^{-2} mol/L, 100 µL), indole-3-pyruvic acid (5.0 x 10^{-2} mol/L, 10 µL), were mixed with EDTA (2.0 x 10^{-3} mol/L, 815 µL) in HEPES buffer (pH = 7.5) at room temperature. The reactions were run for 1h to 24 h. To a 200 µL of the mixture were added 10 µL of a derivatizing solution containing 0.2 M o-phthalaldehyde and 0.2 M *N*-Boc-cysteine/*N*-acetyl-cysteine in methanol, 10 µL of an aqueous buffer solution of 1.0 M K_2HPO_4 (pH 8.0). The resulting solutions were analyzed by HPLC, with UV-vis detection at 344 nm. Standard samples were run before and after the samples to ensure the right retention times of the products. A representative HPLC trace is provided here.



HPLC plot of the formation of D/L tryptophan and D/L alanine



Zoom in of the HPLC plot

Name	Retention Time	Erea
1	23.882	9431
2	24.745	8591
3	70.150	37090
4	71.366	36861

The end