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Supporting Information

First Example of Molecularly Imprinted Nanogels with Aldolase type I activity

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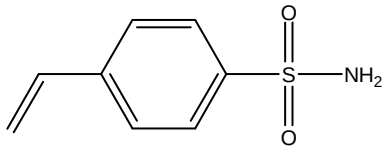
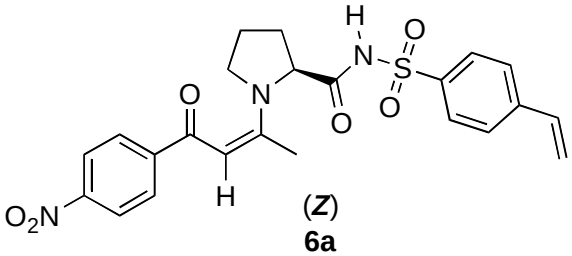
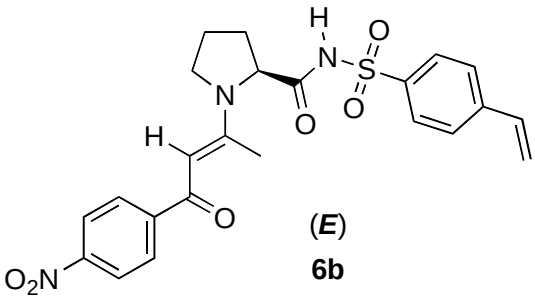
Preparation of Compounds:



4-vinylbenzenesulfonamide: A (3g, 14.6 mmol) quantity of sodium 4-vinylbenzenesulfonate was added portion wise (3 x 1g), at 0˚C under nitrogen, to 15 cm^3 of thionyl chloride. Following addition of 1 cm^3 of dimethyl formamide was left to react at room temperature for 16 h. The reaction was monitored by TLC using diethyl ether/petroleum ether = 6/4 until complete conversion of the starting material. 125 cm^3 of ice was added to the reaction mixture and extracted with diethyl ether. The combined organic phase was dried over $MgSO_4$, filtered and concentrated under vacuum. The unpurified styrensulfonyl chloride was added portion wise, under vigorous stirring, to 50 cm^3 of 30% of aqueous ammonia solution at room temperature. The biphasic mixture was left stirring for 2 h. The mixture was extracted with diethyl ether. The combined organic layers were dried over $MgSO_4$, filtered and concentrated under vacuum to afford (1.54 g, 8.43 mmol) a white solid with a yield of 58%. ^{1}H-NMR (275 MHz, $CDCl_3$) d(ppm): δ = 7.86 (Ar-H, d, 2H, J = 8.55 Hz), 7.52 (Ar-H, d, 2H, J = 8.55 Hz), 6.74 (CH_2=CH-, dd, 1H, J_{CIS} = 11.08 Hz, J_{TRANS} =17.87 Hz), 5.87 (CH_2=CH-, d, 1H, J_{TRANS} =17.87 Hz), 5.42 (CH_2=CH-, d, 1H, J_{CIS} =11.08 Hz), 4.81 (S-NH_2, s, 2H). ^{13}C-NMR (67 MHz, $CDCl_3$) d(ppm) : δ = 139, 135, 127, 117. HR-MS (ESI): Calculated for $C_8H_{13}O_2N_2S$: 201.0692 ($[M+NH4]^+$), found 201.0694. IR (nujol) (S-NH_2) 3340, 3256 cm^{-1}.

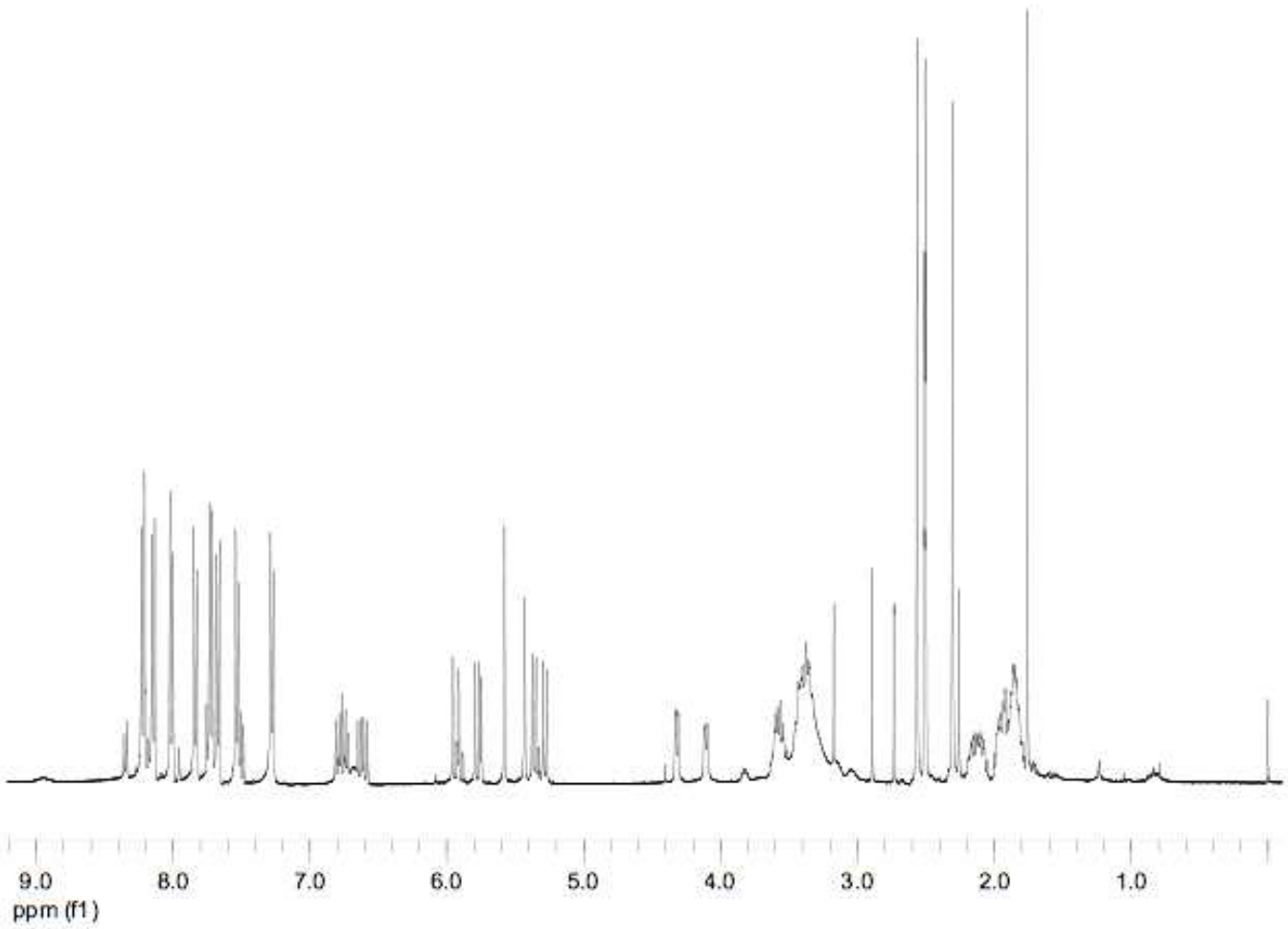


(***Z***)
6a



(***E***)
6b

1-(4-(4-nitrophenyl)-4-oxobut-2-en-2-yl)-N-(4-vinylphenylsulfonyl)pyrrolidine-2-carboxamide (6): A (141 mg, 0.51 mmol) quantity of (5), dissolved in 4 cm^3 of dry dimethylformamide, were added to (105 mg, 0.51 mmol) of (4), under N_2 atmosphere. Activated molecular sieves (0.4 nm) were added to the mixture, which was kept under stirring for 64 h at 40˚C. After 64 h TLC monitoring with dichloromethane/methanol 8/2 showed complete reaction. The reaction mixture was purified with flash chromatography using a mixture dichloromethane/methanol 95/5, without any previous work-up. A (167 mg, 0.36 mmol) quantity of template-monomer complex was recovered giving a yield of 71%. The desired compound is obtained pure as a mixture of the *E* and *Z* geometric isomers. Characterization of the compounds by ^{13}C-NMR. The ^{1}H-NMR and COSY-NMR clearly confirmed the formation of the enaminone. ^{1}H-NMR ($(CD_3)_2SO$, 400 Mhz) (mixture of *E* and *Z* isomers) (see supplementary information). *p*-nitro-Ar : δ = 8.22 (Ar-H, d, 2H, J = 8.00 Hz), 8.01 (Ar-H, d, 2H, J = 8.00 Hz); p-nitro-Ar : 8.14 (Ar-H, d, 2H, J = 8.00 Hz), 7.83 (Ar-H, d, 2H, J = 8.00 Hz); benzenesulfonamide-Ar : 7.72 (Ar-H, d, 2H, J = 8.00 Hz), 7.53 (Ar-H, d, 2H, J = 8.00 Hz); benzenesulfonamide-Ar : 7.67 (Ar-H, d, 2H, J = 8.00 Hz), 7.28 (Ar-H, d, 2H, J = 8.00 Hz); styrenic : 6.78 (CH_2=CH-, dd, 1H, J_{CIS} = 12.00 Hz, J_{TRANS} = 16.00 Hz), 5.93 (CHH=CH-, d, 1H, J_{TRANS}S =16.00 Hz), 5.36 (CH_2=CH-, d, 1H, J_{CIS} = 12.00 Hz); styrenic : 6.61 (CH_2=CH-, dd, 1H, J_{CIS} = 12.00 Hz, J_{TRANS} = 16.00 Hz), 5.77 (CH_2=CH-, d, 1H, J_{TRANS} = 16.00 Hz), 5.28 (CH_2=CH-, d, 1H, J_{CI}S = 12.00 Hz); enaminic proton for major isomer : 5.58 (-C(O)-CH=C-N-, s, 1H); enaminic proton for minor isomer : 5.43 (-C(O)-CH=C-N-, s, 1H); COSY-NMR ($(CD_3)_2SO$, 400 MHz) (mixture of *E* and *Z* isomers) d: (see supplementary information). ^{13}C-NMR: ($(CD_3)_2SO$, 67 MHz) (mixture of *E* and *Z* isomers) d: (183.31/183.02); (175.45/175.12); (163.27/162.82); (148.89/148.60); 144.34; (128.63, 127.53, 126.16, 126.05, 123.85, 123.68); 116.74; (93.05/92.33); (65.31/64.87); (50.00/49.62); (31.09/30.89); (23.75/23.20); 17.87. (see supplementary information) HR-MS (ESI): Calculated for $C_{23}H_{24}O_6N_3S$: 470.1380 ($[M+H]^+$), found 470.1378. m.p: 106˚C.



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18 Feb 2008
Document's Title:
DC111

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D. Carboni DC 111 F6070

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Figure S-1

Figure S-1exp-Aromatic

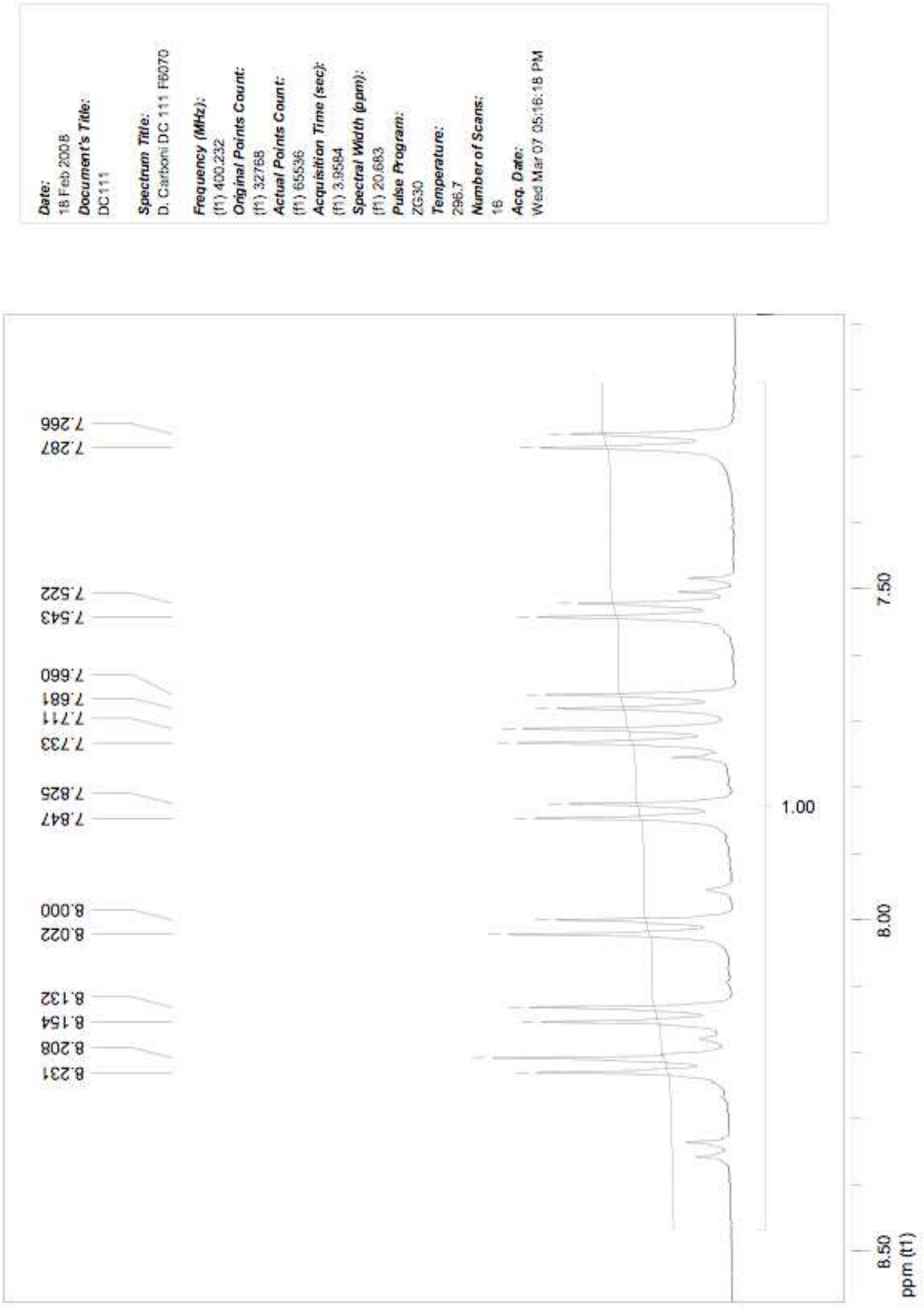


Figure S-1exp-Styrenic

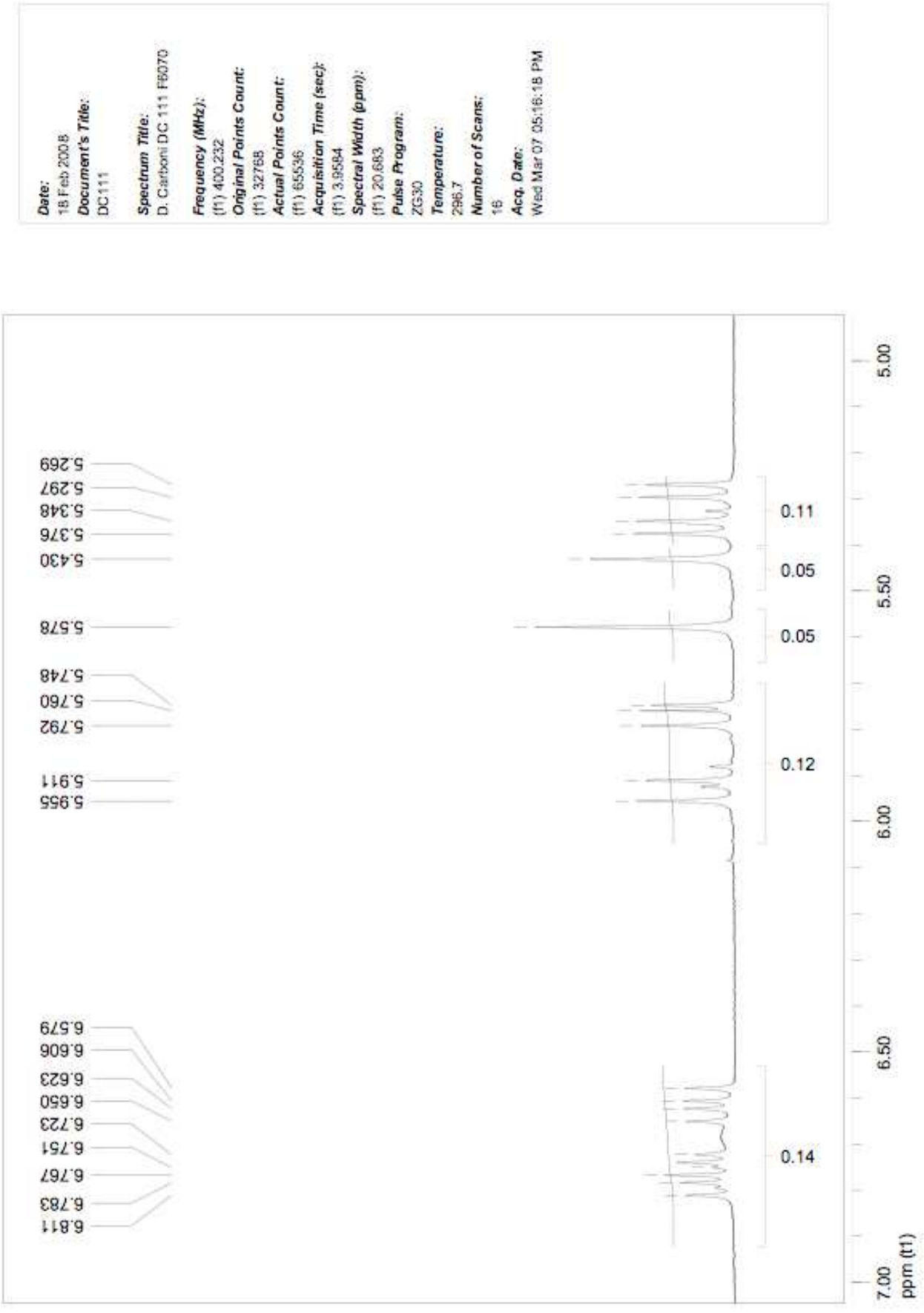


Figure S-1exp-Alifatic

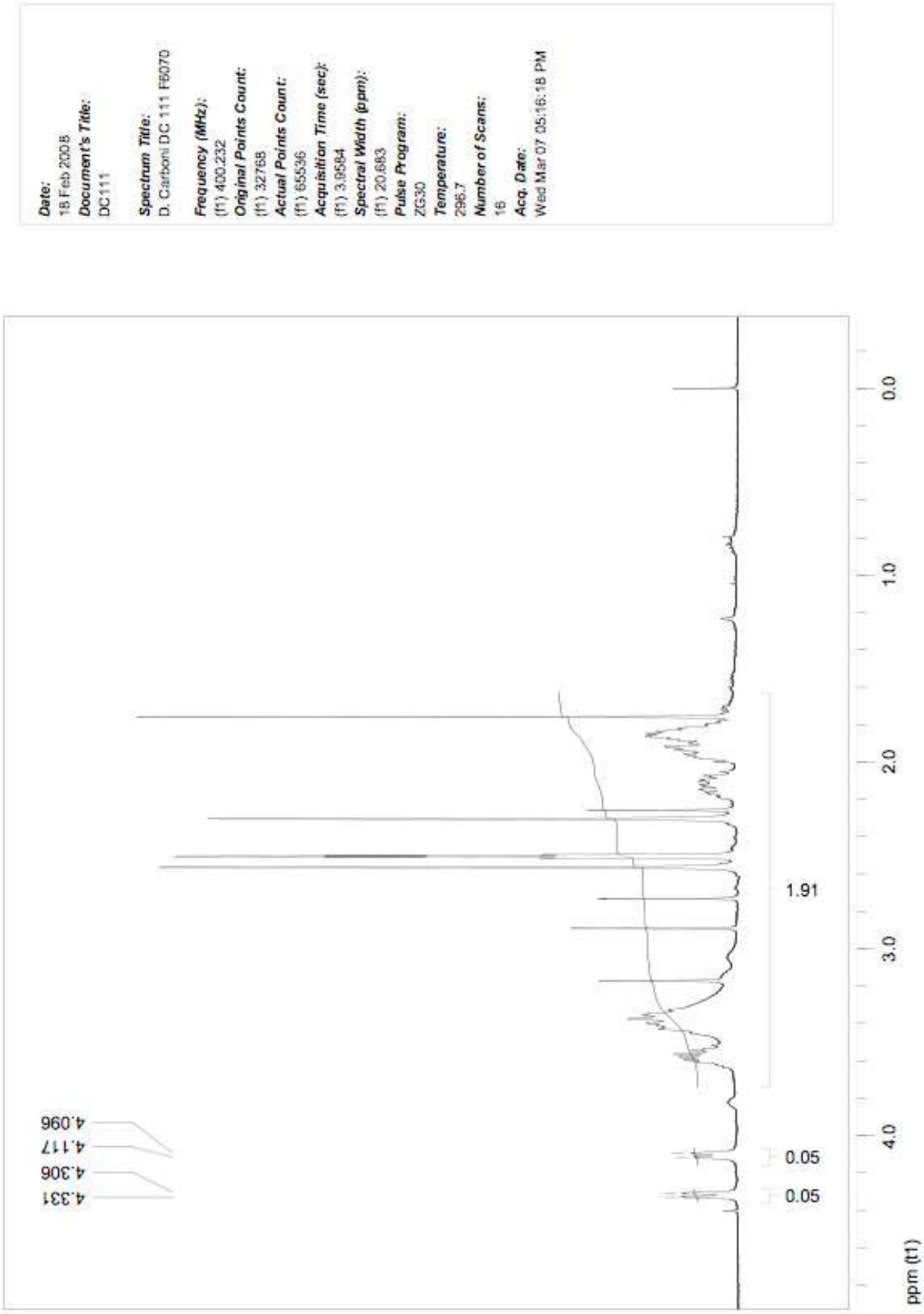


Figure S-2- COSY-NMR

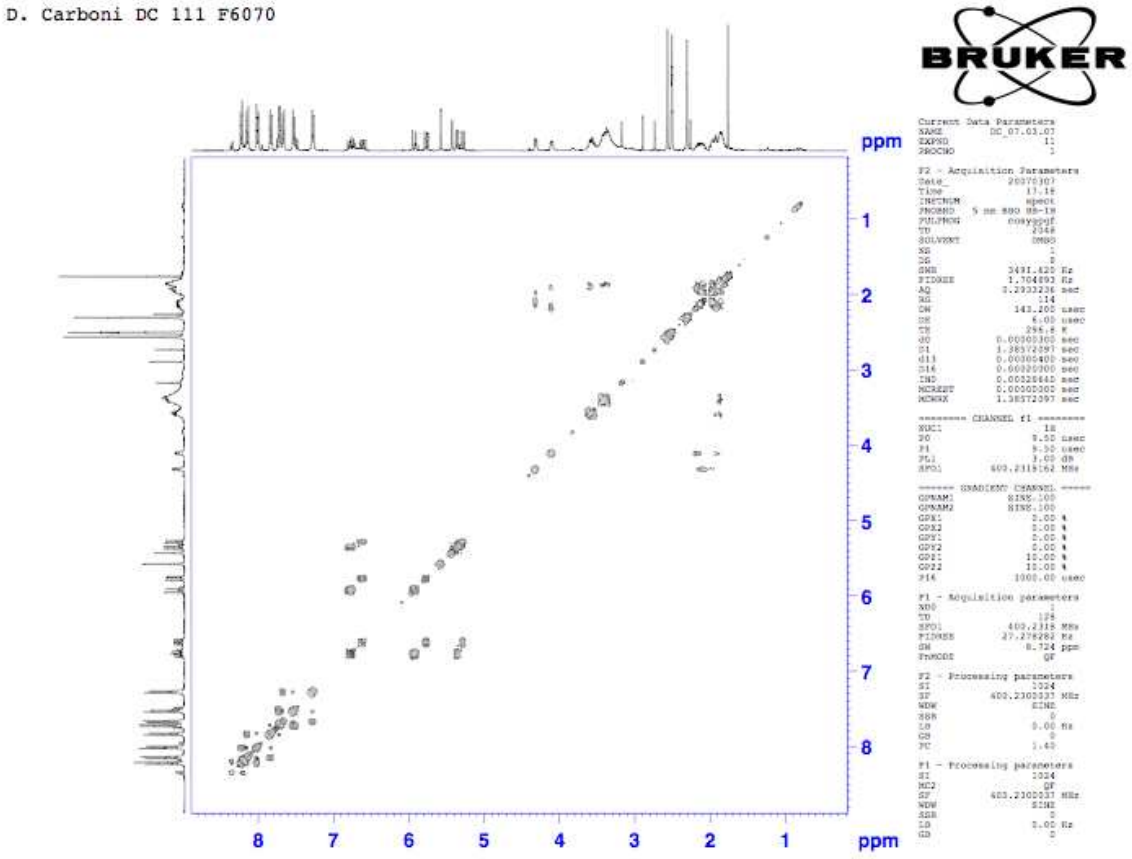
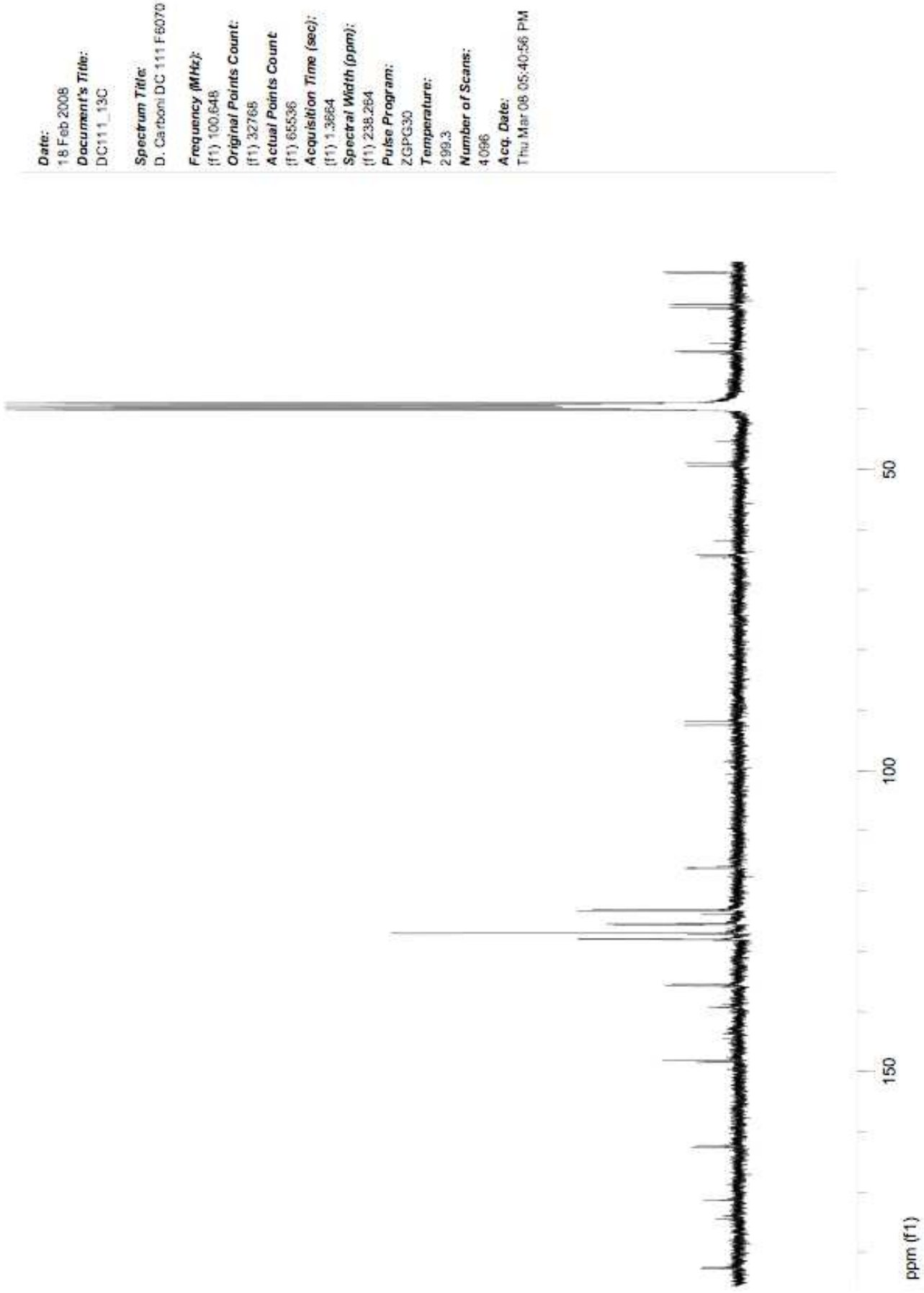


Figure S-3- ^{13}C-NMR



Experimental HPLC procedure:

High performance liquid chromatography (HPLC): Analytical HPLC analysis were performed on a Shimadzu HPLC system (System Controller SCL-10A*vp*, Column Oven CTO-10AS*vp*, by analytical RP-HPLC: Guard Holder HICHROM HI-161 with cartridge HI-5C18-10C, Column HICHROM HI-5C18-250A, 150Å pore size, 5µm particle size, 4.6 x 250 mm, flow rate 1.0 ml/min; solvent A: water Hipersolv with 0.1% trifluoroacetic acid (TFA) (Lancaster); solvent B: acetonitrile (ACN) Hipersolv; Oven temperature 25˚C.

Standard elution was performed with a binary gradient of water (with 0.1% TFA)/ACN and a total flow rate of 1ml/min over 26min, starting with isocratic 30% ACN for the first 5 min, then gradient from 5 to 10 min increasing ACN up to 85%, followed by isocratic from 10 to 14 min and reverse gradient decreasing ACN back to 30% within 18 min. Isocratic was kept at 30% from 18 to 26 min to equilibrate the column for the next injection.

Retention times for the product contained in the reaction mixture:

Reaction of **1** to give **3** : t_R (**2**) = 4.0 min, t_R (**3**) = 9.5 min, t_R (**1**) = 12.6 min, t_R (dehydrated side product) = 13.6 min, t_R (Internal standard) = 14.0 min.

Evaluation of product formation:

To minimise the variability due to HPLC apparatus, concentrations of the aldol product formed during the reaction, were evaluated using an internal standard (methyl 4-nitrobenzoate) 7mM in DMSO. Several samples were prepared containing aldol in concentration from 1 µM to 4 mM. A 400 µl aliquot of each concentration were added to vials containing 20 µl of internal standard. For each concentration 20 µl was injected into HPLC. The ratio between the areas was plotted against the concentration of product so to obtain a straight line, whose slope was used to evaluate product concentrations deriving from the reactions.

General procedure for nanogels catalyzed reactions:

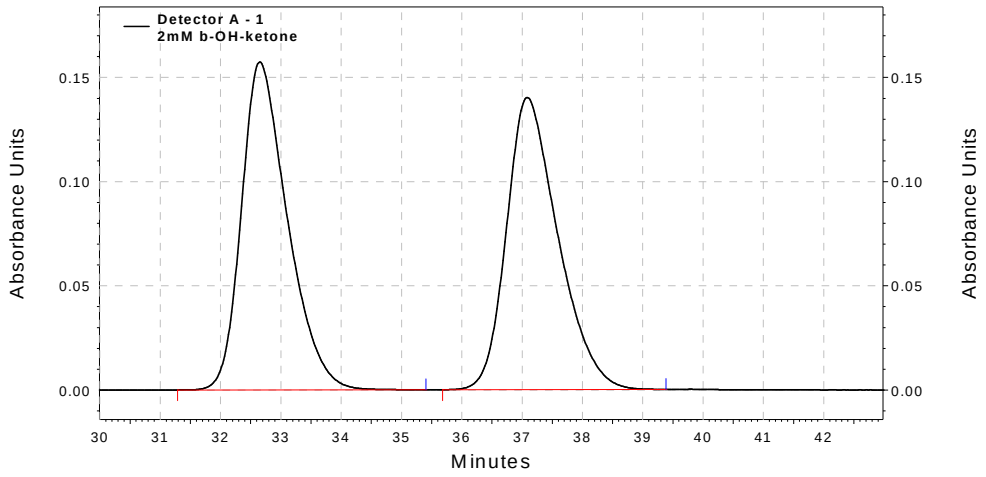
Procedure for the aldol reaction of aldehyde (**1**) with ketone (**2**) at 25˚C: Each reaction mixture was prepared with 60% of DMF, 20% DMSO and 20% acetone. Both the concentration of the nanogel and the ketone donor in the reaction mixtures were kept constant at 0.25 mg/ml and 20% volume, respectively. The final concentration of the acceptor aldehyde in the reaction mixture varied from 0.5 to 8 mM. Reactions were initiated by addition of *p*-NO_2-benzaldehyde to the reaction mixture.
Initial velocities were determined following product formation within 5% of reaction completion. The points were determined experimentally and the best fit value of V_{MAX} and K_m were obtained by fitting the v_i versus $[S]_0$ data to hyperbolic saturation curves by weighted non-linear regression using sigmaplot 8.0 (from SPSS Inc.).

Experiments performed by varying the concentration of catalyst with constant substrates (Figure S-4) concentration showed linear dependence of the initial rate v_i , as would be expected if catalysis was the result of the presence of the nanogels.

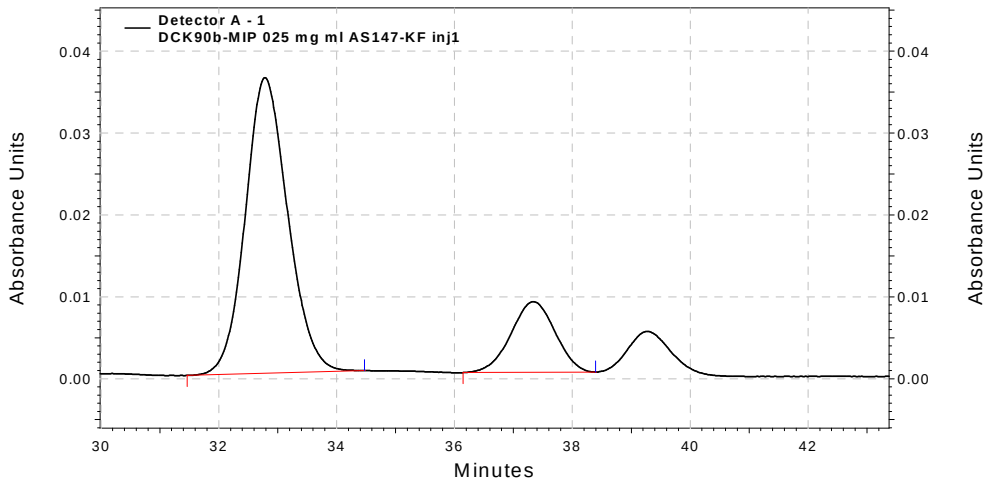
Determination of enantiomeric excess:

Standard analysis was performed in isocratic elution with a mixture of hexane/2-propanol 9/1 and a total flow rate of 1 ml/min over 70 min. Each sample was dissolved in 2-propanol.
t_{maj} (**3**) = 32.6 min, t_{min} (**3**) = 37.1 min.

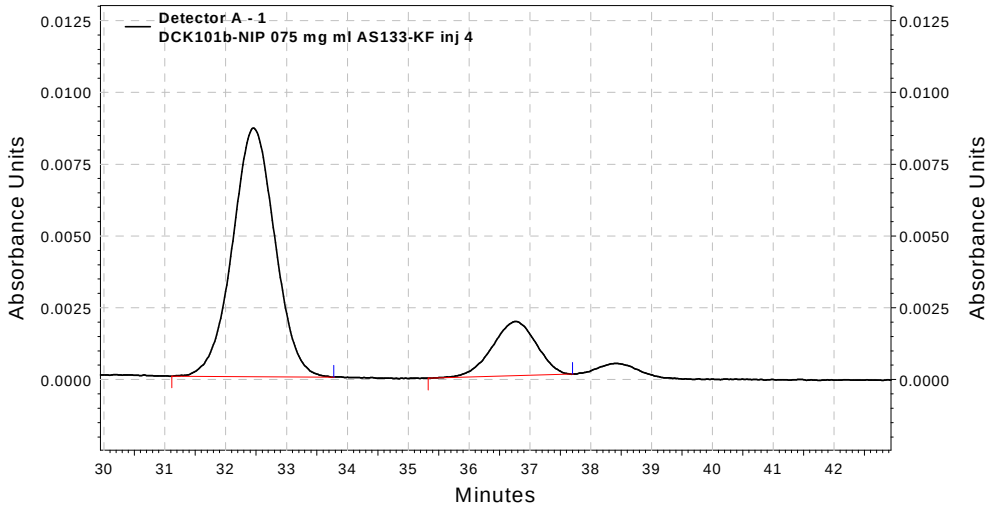
Chromatogram: Racemic mixture



Chromatogram: Imprinted polymer



Chromatogram: Non-imprinted polymer



Notes and References

[1] V. Maggiotti, S. Bahmanyar, M. Reiter, M. Resmini, K. N. Houk and V. Gouverneur, *Tetrahedron*, **2004**, *60*, 619-632.

NMR Prediction:

The ^{13}C NMR spectra were predicted by DFT quantum chemical calculations carried out with the parallel version of Gaussian 03.[1] The geometries of the enaminone **6** were optimized first using the BLYP method [2,3] in combination with the 6-31G(d,p) basis set. The ^{13}C absolute shielding constants were computed using the continuous set of gauge transformations method [4] using the 6-311G(2d,p) basis set. The calculated magnetic shieldings were converted into the δ chemical shifts by noting that at the same level of theory the absolute shielding in tetramethysilane is 175.44.

References:

[1] Gaussian 03, Revision C.02, M. J. Frisch, G. W. Trucks, H. B. Schlegel, G. E. Scuseria, M. A. Robb, J. R. Cheeseman, J. A. Montgomery, Jr., T. Vreven, K. N. Kudin, J. C. Burant, J. M. Millam, S. S. Iyengar, J. Tomasi, V. Barone, B. Mennucci, M. Cossi, G. Scalmani, N. Rega, G. A. Petersson, H. Nakatsuji, M. Hada, M. Ehara, K. Toyota, R. Fukuda, J. Hasegawa, M. Ishida, T. Nakajima, Y. Honda, O. Kitao, H. Nakai, M. Klene, X. Li, J. E. Knox, H. P. Hratchian, J. B. Cross, V. Bakken, C. Adamo, J. Jaramillo, R. Gomperts, R. E. Stratmann, O. Yazyev, A. J. Austin, R. Cammi, C. Pomelli, J. W. Ochterski, P. Y. Ayala, K. Morokuma, G. A. Voth, P. Salvador, J. J. Dannenberg, V. G. Zakrzewski, S. Dapprich, A. D. Daniels, M. C. Strain, O. Farkas, D. K. Malick, A. D. Rabuck, K. Raghavachari, J. B. Foresman, J. V. Ortiz, Q. Cui, A. G. Baboul, S. Clifford, J. Cioslowski, B. B. Stefanov, G. Liu, A. Liashenko, P. Piskorz, I. Komaromi, R. L. Martin, D. J. Fox, T. Keith, M. A. Al-Laham, C. Y. Peng, A. Nanayakkara, M. Challacombe, P. M. W. Gill, B. Johnson, W. Chen, M. W. Wong, C. Gonzalez, and J. A. Pople, Gaussian, Inc., Wallingford CT, 2004.

[2] Becke, A.D., Phys. Rev. 1988, A38, 3098-3100.

[3] Lee, C., Yang, W., Parr, R.G., Phys. Rev. 1988, B41, 785–789.

[4] Keith, T.A., Bader, R.F.W., Chem. Phys. Lett. 1993, 210, 223.