Electronic Supplementary Information

^{13}C-Isotopic labelling for the facilitated NMR analysis of a complex dynamic chemical system

Marta Dal Molin, Giulio Gasparini, Paolo Scrimin, Federico Rastrelli,* Leonard J. Prins*

Department of Chemical Sciences, University of Padova, Via Marzolo 1, 35131 Padova, Italy.

CONTENTS

1. Instrumentation and materials

NMR analysis

For the characterization of the synthesized products, ^{1}H NMR spectra were recorded on a Bruker AC-250 spectrometer operating at 250 MHz or on a Bruker DRX-300 spectrometer operating at 300 MHz. Chemical shifts are reported in ppm using residual solvent $CDCl_3$ (7.26 ppm), CD_3OD (3.31 ppm) or CD_3CN (1.94 ppm) for calibration. ^{13}C NMR spectra, proton decoupled, were recorded at 62.5 MHz using solvent as internal reference $CDCl_3$ (77.16 ppm) or CD_3OD (49 ppm). ^{31}P NMR experiments were performed on a Bruker spectrometer operating at 122 MHz ^{31}P frequency using a solution of H_3PO_4 80% in H_2O as external standard for calibration. For the exchange experiments, all the NMR spectra were recorded on a Varian 400 spectrometer (operating at ^{1}H and ^{13}C frequencies of 400.36 and 100.68 MHz, respectively) or on a Bruker DMX 600 spectrometer (operating at ^{1}H and ^{13}C frequencies of 600.01 and 150.87 MHz, respectively).

The DEPT pulse scheme features both a delay Δ, where antiphase magnetization builds up and a pulse of variable flip angle β for back-conversion of multiple-quantum coherence. It is known that the maximum amplitude of magnetization transfer for methine (CH) carbons occurs for $\beta = 90°$ and $\Delta = 1/(2\ ^{1}J_{CH})$, [J. Keeler, *Understanding NMR Spectroscopy*. 2nd ed.; John Wiley & Sons Ltd.: 2010; p 462-463] whereby a signal enhancement of $|\gamma_H/\gamma_C| \approx 4$ is expected (being γ_S the magnetogyric ratio of nucleus S). In the case of our labelled compounds, a preliminary ^{1}H spectrum easily delivers both the actual $^{1}J_{CH}$ values (as measured from signal splittings) and the optimal β value (via routine calibration procedures). It is also worth noting that the response function of DEPT-90 is quite insensitive to moderate variations of $^{1}J_{CH}$ values. For $^{1}J_{CH}$ discrepancies spanning a range of ±20% about the optimal value, the recovered magnetization exceeds 90%.

MS and LC/MS analysis

The ESI-MS measurements were performed on an Agilent Technologies 1100 Series LC/MSD Trap-SL spectrometer equipped with an ESI source, hexapole filter and ionic trap. The UHPLC/MS measurements were performed on an Agilent 1290 Infinity LC/MS System equipped with an ESI source, quadrupole system and diode array detector.

Purification

Chromatographic purifications were performed using flash chromatography with silica gel Machery-Nagel 230÷400 mesh. HPLC purifications were performed on a preparative HPLC Shimazu LC-8A equipped with a Shimazu SPD-20A UV detector. The column used for separation

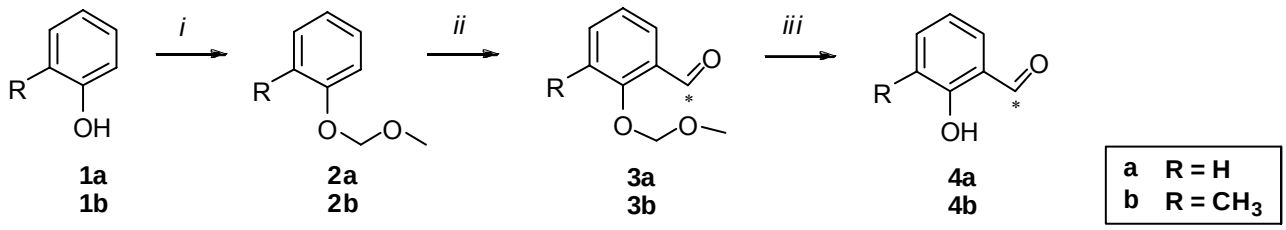
was a Jupiter Proteo 4u 90A 250 x 21.20 mm, 4 μm, flow: 17 mL/min, eluents: H_2O + 0.1% HCOOH (A), CH_3CN + 0.1% HCOOH (B), gradient: 0-15 min 10-90% B. The chromatographic column used for separations in UHPLC analysis was a Zorbax RRHP Eclipse Plus C18 2.1 x 100 mm, 1.8 μm, flow 0.2 mL/min, eluents: H_2O + 0.1% HCOOH (A), CH_3CN + 0.1% HCOOH (B), gradient: 0-5 min 10-90% B. For acqueous phase volume reduction, a reduced pressure centrifuge Genevac EZ-2 plus was used.

Materials

All the reagents used were purchased from Sigma-Aldrich or Acros with >98% purity and were used without any further purifications. Carbonyl-^{13}C-N,N-dimethylformamide (99%) was purchased from Cambridge Isotope Laboratories Inc. All solvents were HPLC-grade and used without further purification. The water used for the MS and LC analysis was filtered with a Millipore MilliQ system. All the deuterated solvents were purchased from Sigma-Aldrich ($CDCl_3$ 99.8%D; CD_3OD-d_4 99.9%D, DMSO-d_6 99.8%D, CD_3CN-d_3 99.8%D).

2. Synthesis and characterization of compounds P_{1-4}

For scaffolds P_{1-3} a general synthetic scheme was used relying on the initial preparation of the ^{13}C-labeled salicyl aldehyde derivatives **4a-b** (Scheme SI-1). The final products P_{1-3} were obtained from **4a-b** in 1 or 2 steps (Schemes SI-2-4). For the synthesis of P_4 an alternative synthetic procedure was followed (Scheme SI-5).



Scheme SI-1: i) NaH, MOM-Cl, THF, ii) n-BuLi, ^{13}C-DMF, Et_2O, iii) HCl in Et_2O.

2.1 General procedure for the synthesis of compounds 2

A suspension of NaH (60% mineral oil, 1.5 eq) in dry THF (10 mL) was cooled to 0°C, after which a solution of the corresponding commercial available phenol (1 eq) in dry THF (3 mL) was added dropwise. The mixture was stirred for 15 min at 0°C, then heated to room temperature and stirred for additional 30 min. After cooling to 0 °C, MOM-Cl (1.8 eq) was added dropwise and the mixture was stirred for 3 hours. The reaction mixture was washed with saturated NH_4Cl (3 x 25 mL) and extracted with EtOAc (3 x 25 mL). The combined organic layers were dried over anhydrous Na_2SO_4 and the solvent was evaporated under reduced pressure to give a yellow oil. The residue was purified with flash chromatography to obtain the desired product as a colourless liquid.

Compound 2a. Yield 57% **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 3.69 (s, 3H), 5.38 (s, 2H), 7.35 (m, 5H). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 55.95, 94.63, 116.21, 121.85, 129.44, 157.18.
Compound 2b. Yield 62% **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 2.38 (s, 3H), 3.61 (s, 3H), 5.33 (s, 2H), 7.18 (m, 4H). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 16.25, 55.95, 94.46, 113.86, 121.57, 126.8, 127.36, 130.78, 155.38.

2.2 General procedure for the synthesis of compounds 3

n-BuLi (1.2 eq) was added dropwise to a solution of **2** in dry ether (20 mL) at room temperature. The resulting solution was stirred for 1 hour after which ^{13}C-DMF (1.5 eq) was added dropwise. The mixture was stirred for an additional hour, after which it was washed with saturated NH_4Cl (3 x 25 mL). The solution was extracted with diethyl ether (3 x 25 mL) and the combined organic layers

were dried over anhydrous Na_2SO_4. Evaporation of the solvent under reduced pressure gave a yellow oil. After purification by flash column chromatography (SiO_2, petroleum ether/ ethyl acetate 95/5) compound **3** was obtained as a yellow oil.

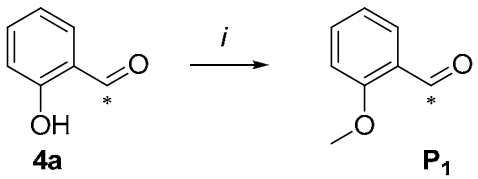
Compound 3a: **Yield** 57%. **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 3.55 (s, 3H), 5.33 (s, 2H), 7.35 (m, 3H), 7.87 (ddd, 1H, J=1.8Hz, J=4.2Hz, J=7.7Hz), 10.53 (d, 1H, J=181.1Hz). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) ppm 189.70. **ESI-MS** (+, ACN (0.1% HCOOH)): *m/z* 190.3 $[M+Na]^+$,calcd: 190.2, 206.2 $[M+K]^+$, calcd: 206.2.
Compound 3b. Yield 63%. **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 2.35 (s, 3H), 3.59 (s, 3H), 5.07 (s, 2H), 7.16 (t, 1H, J=7.6Hz), 7.45 (d, 1H, J=7.5Hz), 7.69 (ddd, 1H, J=1.6Hz, J=4.3Hz, J=6.4Hz), 10.32 (d, 1H, J=180.0Hz). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 191.01. **ESI-MS** (+, ACN (0.1% HCOOH)):*m/z* 204.2 $[M+Na]^+$, calcd: 204.2.

2.3 General procedure for the synthesis of compounds 4

Compound **3** was dissolved in a solution of HCl in ether (1 M, 2 mL). The solution was stirred for 24 hours, after which the solvent was removed under reduced pressure yielding a red residue. The compound was purified by flash column chromatography (SiO_2, CH_2Cl_2) to give **4** as a colorless oil.

Compound 4a. Yield 87 %. **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm), 6.98-7.05 (m, 2H), 7.50-7.59 (m, 2H), 9.90 (d, 1H, J=176.3Hz), 11.03 (s, 1H). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 196.49.
Compound 4b. Yield 90%. **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 2.27 (s, 3H), 6.93 (t, 1H, 7.5 Hz), 7.38-7.43 (m, 2H) , 9.87 (d, 1H, J=175.9Hz), 11.27 (s, 1H). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 196.73.

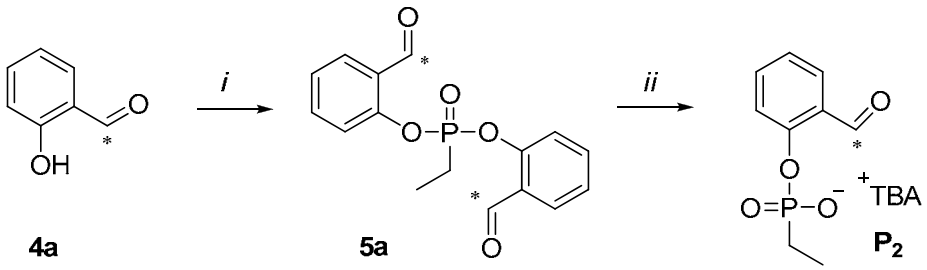
2.4 Synthesis of compound P_1



Scheme SI-2: i) CH_3I, CH_3CN, 40°C.

Compound 4a (20 mg, 0.15 mmol) was dissolved in CH_3CN (2 mL) in a sealed pyrex vial with a screw cap. Methyl iodide (300 µL, 4.8 mmol, 12 eq) and K_2CO_3 (large excess) were then added and the resulting mixture was stirred at 40°C for 7 hours. The mixture was concentrated under vacuum and the resulting residue was treated with CH_2Cl_2 and filtered. The solvent was evaporated under reduced pressure to give $\mathbf{P_1}$ as a pale yellow solid (11.5 mg, 57% yield). **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 3.93 (s, 3H), 7.32 (m, 3H), 7.83 (m, 1H), 10.47 (d, 1H, J=180.5Hz). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 189.81. **ESI-MS** (+, ACN (0.1% HCOOH)):*m/z* 176.3 $[M+K]^+$, calcd: 176.2.

2.5 Synthesis of compound P_2



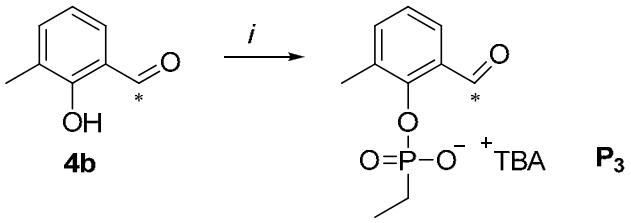
Scheme SI-3: i) NaH, $EtPOCl_2$, THF, ii) NaOH, acetone/H_2O, TBA^+AcO^- in $CHCl_3$.

Compound 5a: A suspension of NaH (60% mineral oil, 33.6 mg, 0.84 mmol) in dry THF (20 mL) was cooled to 0°C, after which a solution of compound (**4a**) (70 mg, 0.57 mmol) in THF (3 mL) was added dropwise. The resulting solution was stirred for 2 hours at room temperature and then cooled again to 0°C, after which $EtPOCl_2$ (31 µL, 0.29 mmol) was added dropwise. The mixture was stirred overnight and then washed with distilled water (3 x 25 mL). The mixture was then extracted with CH_2Cl_2 (3 x 20 mL) and the organic layer was dried over anhydrous Na_2SO_4. After evaporation of the solvent under vacuum a yellow solid was obtained, which was recrystallized from CH_3CN to give **5a** as white crystals (46 mg, 50% yield). **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 1.30 (td, 3H, J=7.7Hz, J=22.0Hz), 2.19 (qd, 2H, J=7.6Hz, J=18.2Hz), 7.15-7.76 (m, 8H), 10.17 (d, 2H, J=181.2Hz). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 188.01. **^{31}P-NMR** (122 MHz, $CDCl_3$) proton decoupled δ (ppm): 28.94. **ESI-MS** (+, ACN (0.1% HCOOH)): *m/z* 198.3 $[M-C_7H_5O_2]^+$,calcd: 198.2, 321.2 $[M+H]^+$, calcd: 321.2, 343.4 $[M+Na]^+$.calcd: 343.2.

Compound P_2: Compound **5a** (46 mg, 0.14 mmol) was dissolved in acetone (3 mL) and the solution was cooled to 0°C. A solution of NaOH (0.1 N, 1.43 mL, 0.143 mmol) was added and the

mixture was stirred for 10 hours. MilliQ water (3 mL) was then added and the obtained solution was extracted with CH_2Cl_2 (4 x 10 mL). The aqueous layer was lyophilized giving a white solid (13 mg, 38% yield). The compound was then dissolved in MilliQ water (3 mL) and a solution of tetrabutylammonium acetate (16.2 mg, 0.05 mmol) in $CHCl_3$ (2.7 mL) was added. The mixture was stirred overnight after which the acqueous layer was lyophilized to give a brown solid which was treated with CH_2Cl_2 and filtered. The solvent was evaporated under reduced pressure obtaining a yellow oil (20.0 mg, 85 % yield). **^{1}H-NMR** (250 MHz, CD_3CN) δ (ppm) 0.96 (t, 12H, J=7.3Hz), 1.08 (td, 3H, J=7.5Hz, J=18.7Hz), 1.34 (qd, 8H, J=7.3Hz, J=14.5Hz), 1.41-1.66 (m, 10H) , 3.06-3.13 (m, 8H) , 7.05 (t, 1H, 7.0 Hz), 7.46-7.55 (m, 2H), 7.68-7.71 (m, 1H), 9.88 (d, 1H, J=93.7Hz). **^{13}C-NMR** (62.5 MHz, CD_3CN) δ (ppm): 191.62. **^{31}P-NMR** (122 MHz, $CDCl_3$) proton decoupled δ (ppm): 34.43. **UHPLC** (Zorbax RRHP Eclipse Plus C18 2.1 x 100 mm, 1.8 µL, flow 0.2 mL/min, eluents: H_2O + 0.1% HCOOH (A), CH_3CN + 0.1% HCOOH (B), gradient: 0-5 min 10-90% B): 2.7 min, **ESI** : (-, ACN): *m/z* 214.3 $[M]^-$.calcd: 214.1.

2.6 Synthesis of compound P_3

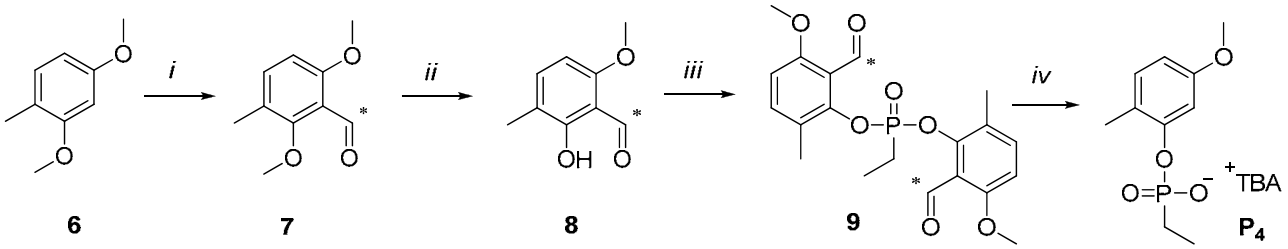


Scheme SI-4: i) NaH, $EtPOCl_2$, THF.

A solution of compound **4b** (133 mg, 0.97 mmol, 1 eq) in dry THF (10 mL) was added dropwise to a suspension of NaH (60% mineral oil, 54 mg, 1.35 mmol, 1.4 eq) in dry THF (1 mL) at 0 °C. The solution was stirred at room temperature for 2 hours and then cooled again to 0 °C, after which $EtPOCl_2$ (165 uL, 1.54 mmol) was added dropwise. The mixture was stirred at room temperature for 2 hours and then 3 mL of MilliQ water were added. The solution was concentrated with vacuum centrifugation after which the acqueous layer was separated and purified with preparative HPLC (Jupiter Proteo 4u 90A 250 x 21.20 mm, 4 µm, flow: 17 mL/min, eluents: H_2O + 0.1% HCOOH (A), CH_3CN + 0.1% HCOOH (B), gradient: 0-15 min 10-90% B, λ= 280 nm: 10.03 min) obtaining after lyophilization a white solid (11.3 mg, 0.05 mmol, 54% yield based on conversion). The product was then dissolved in MilliQ water (3 mL) and a solution of NaOH (0.1N, 0.5 mL, 1 eq) was added in order to obtain the sodium salt. A solution of tetrabutylammonium acetate in $CHCl_3$ (2.3 mL, 1 eq) was then added and the resulting mixture was stirred overnight. The mixture was

filtered and the solvent was removed under vacuum to afford the desired product as a hygroscopic solid. 22.8 mg, 94% yield. Compound **P_3** was stored under an inert atmosphere. **^{1}H-NMR** (250 MHz, CD_3CN) δ (ppm) 0.95 (t, 12H, J=7.3Hz), 1.14 (td, 3H, J=7.6Hz, J=18.5Hz), 1.34 (qd, 8H, J=7.3Hz, J=14.6Hz), 1.60 (m, 10H), 2.36 (s, 3H), 3.07 (dd, 8H, J=7.1Hz, J=9.9Hz), 7.05 (t, 1H, J=7.6Hz), 7.43 (d, 1H, J=7.3Hz), 7.56-7.59 (m, 1H), 10.38 (d, 1H, J=184.9Hz). **^{13}C-NMR** (62.5MHz, $CDCl_3$) δ (ppm) 189.41. **^{31}P-NMR** (122 MHz, CD_3CN) proton decoupled δ (ppm): 24.19. **ESI-MS** (+, ACN (0.1% HCOOH)): *m/z* 230.1 $[M+2H]^+$, calcd: 230.1, 252.3 $[M+Na+H]^+$, calcd: 252.1.

2.7 Synthesis of compound P_4



Scheme SI-5: i) n-BuLi, ^{13}C-DMF, THF, ii) $MgI_2(Et_2O)$, Et_2O/toluene, 60°C, iii) NaH, $EtPOCl_2$, THF, iv) NaOH, acetone/H_2O, TBA^+AcO^- in $CHCl_3$.

Compound 7: A solution of n-BuLi (1.6 M in hexane, 4.8 mmol, 1.6 eq) was added dropwise to a solution of 2,4-dimethoxytoluene **6** (627 mg, 4.12 mmol, 1eq) in dry ether (10 mL).The mixture was stirred for 2 hours after which ^{13}C-DMF (390 uL, 4.80 mmol, 1.2 eq) was added dropwise. The mixture was then stirred for 2 hours and then washed with a saturated acqueous solution of NH_4Cl (3 x 20mL). The combined organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure to obtain a yellow oil, which was purified with flash column chromatography (SiO_2; CH_2Cl_2) yielding a yellow oil (391.2 mg, 52% yield). **^{1}H-NMR** (200 MHz, $CDCl_3$) δ (ppm) 2.22 (s, 3H), 3.81 (s, 3H), 3.87 (s, 3H), 6.66 (dd, 1H, J=1.3Hz, J=8.6Hz), 7.33 (d, 1H, J=8.6Hz), 10.45 (d, 1H, J=181.5Hz). **^{13}C-NMR** (62.5MHz, $CDCl_3$) δ (ppm) 189.72. **ESI-MS** (+, ACN (0.1% HCOOH)): *m/z* 182.2 $[M+H]^+$, calcd: 182.2, 204.1 $[M+Na]^+$, calcd: 204.2.

Compound 8[1]: To a solution of magnesium iodide etherate in toluene, prepared from magnesium (156 mg, 6.48 mmol) and crystalline iodine (666 mg, 2.62 mmol) in a 4:6 mixture of dry ether and

[1] Yamaguchi S., Nedachi M., Yokoyama H., Hirai Y., *Tetrahedron Lett*. **1999**, *40*, 7363-7365.

dry toluene (15 mL), was added a solution of compound **7** (391.2 mg, 2.16 mmol) in dry toluene (3 mL). The mixture was stirred overnight at 60 °C. After cooling to room temperature, HCl (10%) was added and the mixture was extracted with diethyl ether (4 x 25 mL). The solvent was evaporated under reduced pressure to give a black residue which was treated with a solution of sodium thiosulfate (0.1 M solution, 4 x 20 mL) to remove iodine. The mixture was then extracted with ether (3 x 25 mL) and the combined organic layers were dried over anhydrous Na_2SO_4 and concentrated to dryness to give a yellow solid. After purification by column chromatography (SiO_2, petroleum ether/CH_2Cl_2 7/3) compound **8** was obtained as a yellow solid (166.3 mg, 46%). **^{1}H-NMR** (200 MHz, $CDCl_3$) δ (ppm) 2.15 (s, 3H), 3.86 (s, 3H), 6.28 (dd, 1H, J=1.7Hz, J=8.4Hz), 7.26 (d, 1H, J=8.3Hz), 10.33 (d, 1H, J=182.8Hz), 12.22 (s, 1H). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 194.49.

Compound 9: A solution of compound **8** (92 mg, 0.55 mmol, 1 eq) in THF (3 mL) was added dropwise to a suspension of NaH (60% mineral oil, 24.6 mg, 0.62 mmol, 1.1 eq) in anhydrous THF (10 mL) at 0°C. The resulting solution was stirred at room temperature for 2 hours and cooled again to 0°C, after which $EtPOCl_2$ (30 µL, 0.31 mmol) was added. The mixture was stirred overnight, after which distilled water (15 mL) was added. The mixture was then extracted with EtOAc (4 x 25 mL), and the organic layer was dried over anhydrous Na_2SO_4 and evaporated to dryness. The residue was treated with a mixture of $CHCl_3$/hexane obtaining compound **9** as white crystals (19.6 mg, 16% yield, first crop). **^{1}H-NMR** (250 MHz, $CDCl_3$) δ (ppm) 1.31 (td, 3H, J=7.7Hz, J=22.0Hz), 2.11-2.36 (m, 8H), 3.86 (s, 6H), 6.74 (d, 2H, J=8.7Hz), 7.32 (d, 2H, J=8.6Hz), 10.38 (d, 2H, J=184.8Hz). **^{13}C-NMR** (62.5 MHz, $CDCl_3$) δ (ppm) 189.07. **ESI-MS** (+, ACN (0.1% HCOOH)): *m/z* 431.4 $[M+Na]^+$, calcd: 431.4.

Compound P_4: Compound **9** (20 mg, 0.048 mmol) was dissolved in acetone (4 mL) and cooled to 0 °C. A solution of NaOH (0.1 N) was added (0.48 mL, 0.048 mmol) and stirring was continued for 10 hours. Distilled water (5 mL) was then added and the solution was extracted with CH_2Cl_2.(5 x 15 mL). The acqueous layer was lyophilized obtaining the sodium salt of **P_4** as a white solid. The product was then suspended in $CHCl_3$ (5 mL) and a solution of tetrabutylammonium acetate (14.4 mg, 0.048 mmol) in $CHCl_3$ (2.9 mL) was added. The mixture was stirred overnight, then filtered and the solvent was removed under vacuum to obtain a pale yellow oil. (15 mg, 62% yield). **^{1}H-NMR** (300 MHz, CD_3CN) δ (ppm) 0.97 (t, 12H, J=7.3Hz), 1.11 (td, 3H, J=7.6Hz, J=17.8Hz), 1.35 (qd, 8H, J=7.3Hz, J=14.4Hz), 1.56 (m, 10H), 2.28 (s, 3H), 3.08 (m, 8H), 3.77 (s, 3H), 6.62 (d, 1H, J=8.4Hz), 7.27 (d, 1H, J=8.5Hz), 10.37 (d, 1H, J=184.0Hz). **^{13}C-NMR** (62.5 MHz, DMSO-d_6) δ

(ppm) 190.67. **^{31}P-NMR** (122 MHz, $CDCl_3$) proton decoupled δ (ppm): 29.28. **ESI-MS** (-, CH_3CN): *m/z* 258.2 $[M]^-$, calcd: 258.2.

3. General procedure used for signal assignment

Stock solutions of the scaffold molecules $\mathbf{P_{1\text{-}4}}$ were prepared in CD_3CN with concentrations ranging from 30 to 70 mM The exact concentrations of the scaffold stock solutions were determined using ^{13}C-DMF as internal standard. The hydrazide/amine stock solutions were prepared in CD_3OD with known concentrations around 200 mM. The hydrazide/amine stock solutions were diluted in an NMR tube with CD_3OD. The scaffold solutions were then added sequentially causing the immediate formation of the corresponding hydrazone/immine. The DEPT spectrum was collected after every addition on a Varian 400 MHz spectrometer at 298 K. After the addition of all the scaffolds, CD_3OD was added to bring the total volume at 600 µL. After all the scaffolds were added, the newly formed hydrazones/imines were present in a concentration around 2.5 mM each, depending on the exact amount of the corresponding added scaffold ($\mathbf{P_1}$: 2.22 mM, $\mathbf{P_2}$: 2.28 mM; $\mathbf{P_3}$: 2.23 mM; $\mathbf{P_4}$ 2.24 mM). The final concentration of hydrazide/imine was 25 mM each in the 600 µL mixture. It is worth noting that every hydrazone gives a set of at least 2 signals based on the numbers of formed isomers, whose intensity corresponds to the isomer ratio as showed in previous experiments[2] , while all the imines give a unique signal. The NMR addition experiments used for assigning signals to hydrazones $\mathbf{P_{1\text{-}4}H+}$, and imines $\mathbf{P_{1\text{-}4}A}$ and $\mathbf{P_{1\text{-}4}A+}$ are given in Figures SI-1-4. The analogous assignment for hydrazones $\mathbf{P_{1\text{-}4}H}$ is given in the manuscript.

[2] Gasparini G., Vitorge B., Scrimin P., Jeannerat D., Prins L. J., *Chem. Commun*. **2008**, *26*, 3034-3036.

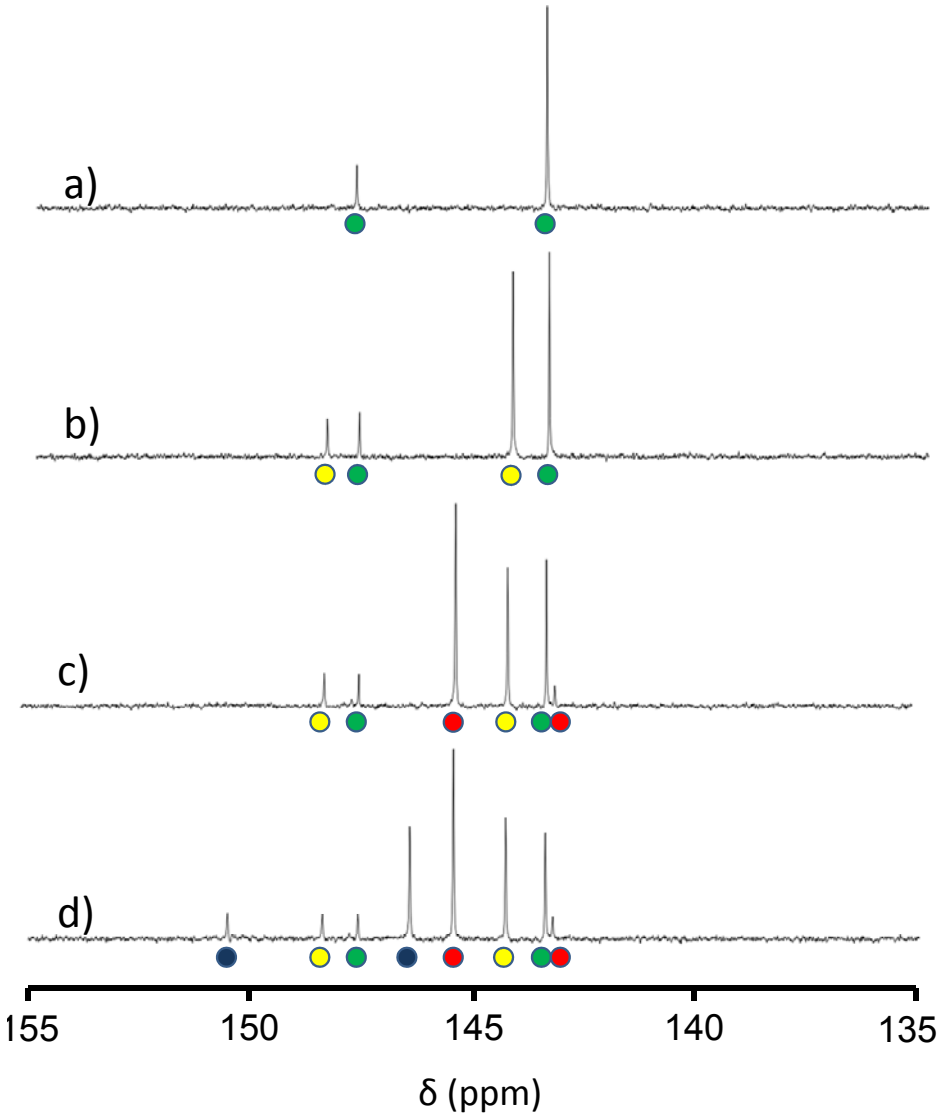


Figure SI-1: Signal assignment based on the sequential addition of scaffolds $\mathbf{P_1}$ – $\mathbf{P_4}$ (2.5 mM each) to a solution of hydrazide **H+** (25 mM) in CD_3OD at 298K (400 MHz). Scaffolds were added in the following order: a) $\mathbf{P_1}$ (●) ;b) $\mathbf{P_2}$ (●);c) $\mathbf{P_4}$ (●); d) $\mathbf{P_3}$ (●).

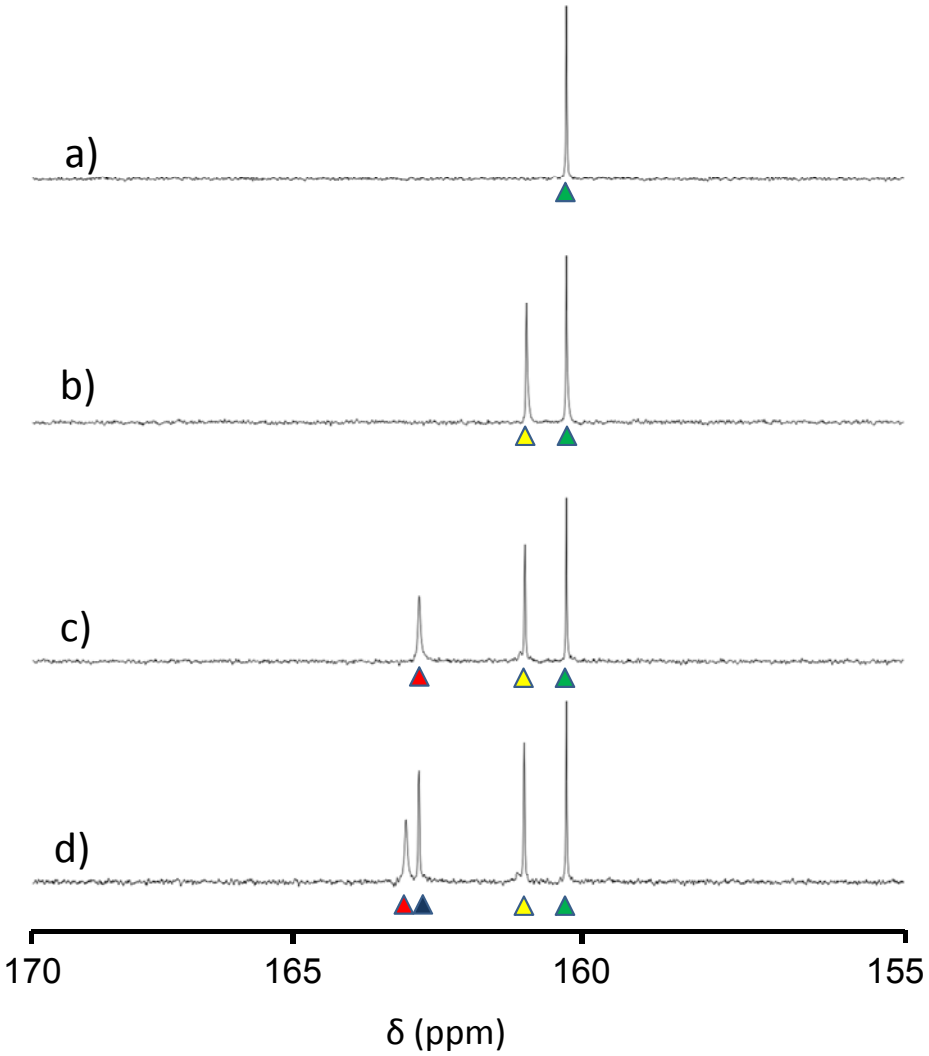


Figure SI-2: Signal assignment based on the sequential addition of scaffolds $\mathbf{P_1}$ – $\mathbf{P_4}$ (2.5 mM each) to a solution of amine **A** (25 mM) in CD_3OD at 298K (400 MHz). Scaffolds were added in the following order: a) $\mathbf{P_1}$ (▲) ;b) $\mathbf{P_2}$ (▲);c) $\mathbf{P_4}$ (▲); d) $\mathbf{P_3}$ (▲).

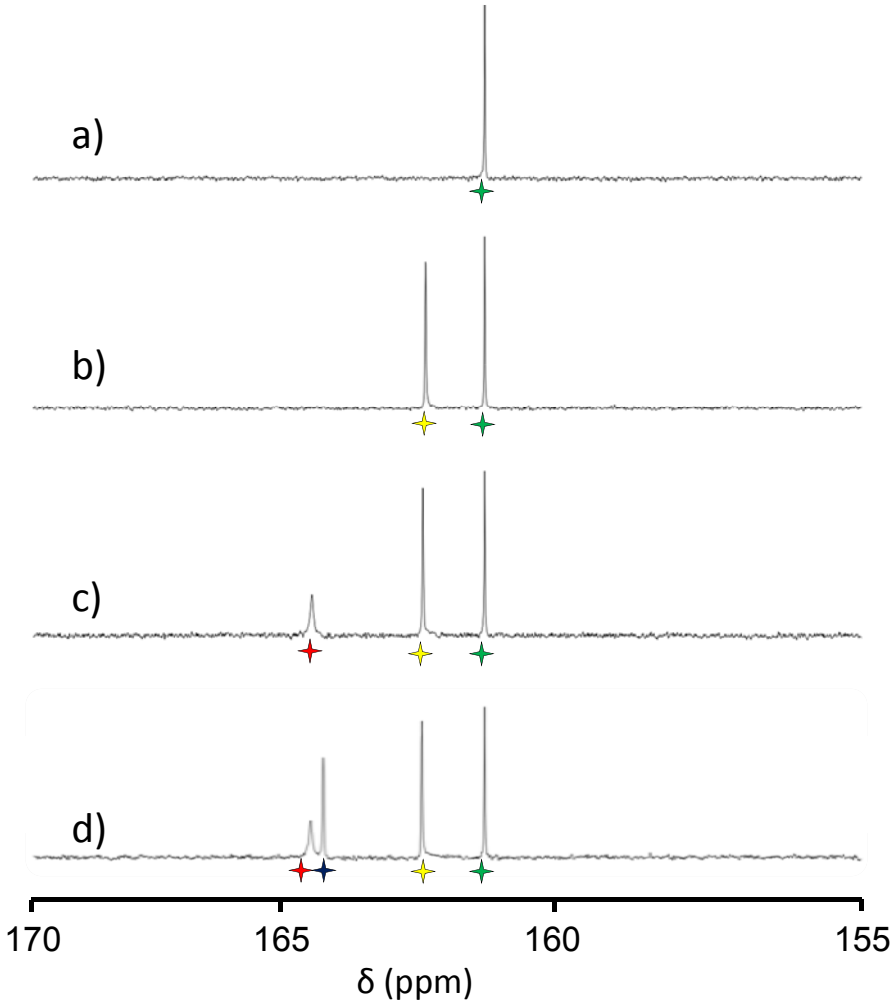


Figure SI-3: Signal assignment based on the sequential addition of scaffolds $\mathbf{P_1}$ – $\mathbf{P_4}$ (2.5 mM each) to a solution of amine **A** (25 mM) in CD_3OD at 298K (400 MHz). Scaffolds were added in the following order: a) $\mathbf{P_1}$ (★) ;b) $\mathbf{P_2}$ (★);c) $\mathbf{P_4}$ (★); d) $\mathbf{P_3}$ (★).

4. General procedure used for the competition experiments between hydrazides H/H+ or amines A/A+.

In an NMR tube, equal volumes of the separate hydrazide/imine libraries (see previous paragraphs) were mixed together in order to obtain a solution containing all the scaffolds $\mathbf{P_{1\text{-}4}}$ and the desired couple of hydrazides **H-H+** or amines **A-A+**. In this manner, the concentration of every scaffold $\mathbf{P_{1\text{-}4}}$ in the final mixture was equal to the one obtained during the experiments of signal assignment (around 2.5 mM for each compound, see previous paragraph) while the concentration of hydrazides/amine resulted 12.5 mM each. After the mixing of the two libraries in the tube, some signals experimented a shift due to the change of ionic strength, which complicated the assignment. To overcome this problem, the second solution was added in two steps, By comparing the spectra obtained after the two additions it was possible to distinguish the signals originating from the addition of the second library. The mixtures were kept at 300 K until thermodynamic equilibrium was reached, indicated by the absence of further changes in the NMR spectrum (2 days for hydrazones and 8 days for imines[3]). A subsequent addition of a catalytic amount of TFA (0.1%) did not change the distribution obtained after this time. Once the equilibrium was reached, DEPT experiments were performed on a Bruker 600 spectrometer, using CD_3OD as internal reference (49 ppm) (Figure SI-4) and a delay $\Delta = 2.7$ ms. The concentration of each member was evaluated by the integration of the signals relative to each compound. The equilibrium constant K_{eq} and $\Delta G°(T=298K)$ relative to the exchange reaction were calculated separately for every scaffold (Tables SI-1-3).

[3] Only for the couple $\mathbf{P_4A/A+}$, the equilibrium was not reached. For this reason the measurement on the relative stability of these species was performed in a different manner (see next paragraph).

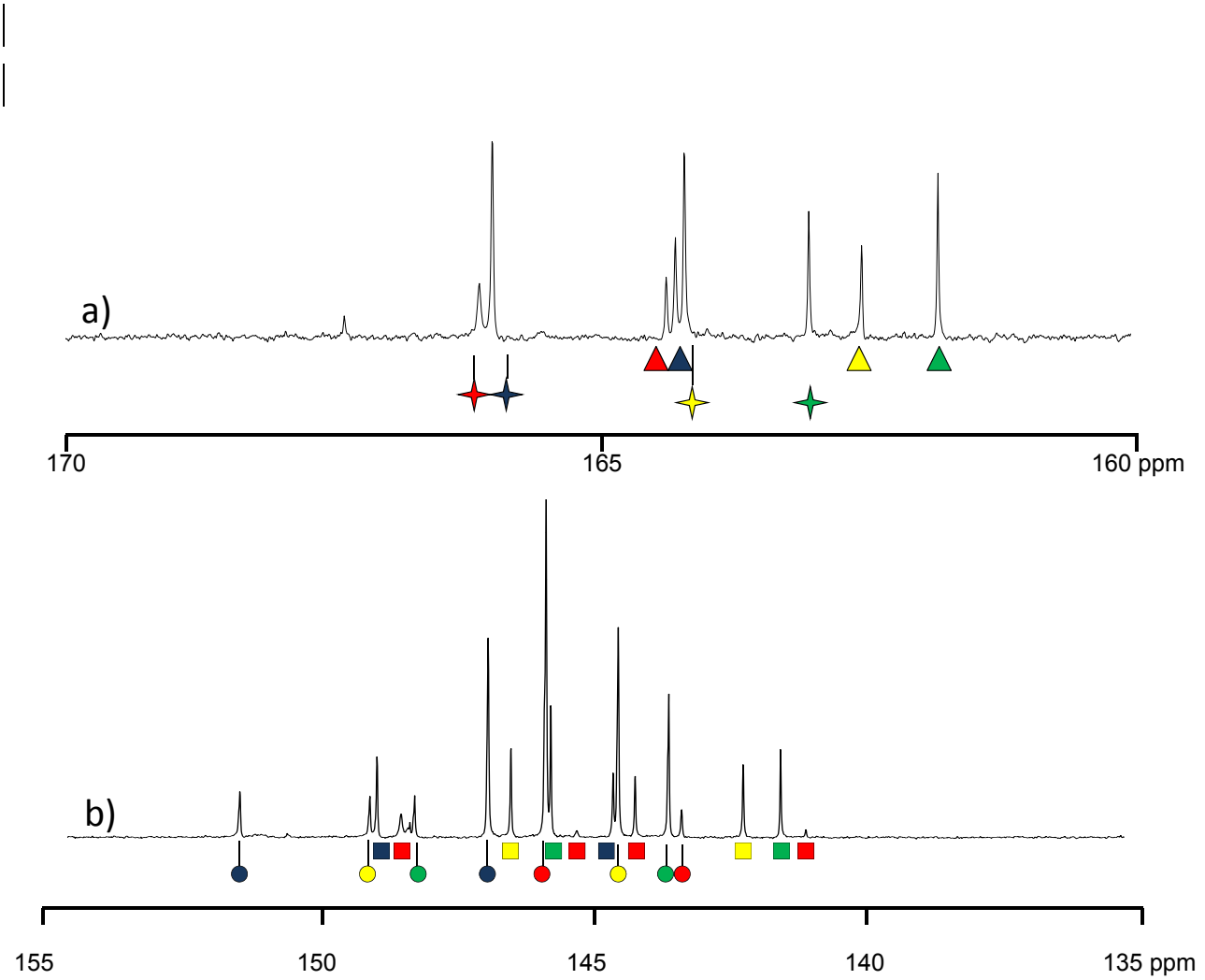


Figure SI-4: DEPT spectra (400 MHz, CD_3OD, 298 K) of a) the mixtures of imines and b) hydrazones at thermodynamic equilibrium.

Table SI-1 : Concentration of hydrazones obtained from the integration of the signals of the mixture of hydrazones (Figure SI-4b).

		P_xH				P_xH+		Sum
		Isomer max		*Isomer min*		*Isomer max*	*Isomer min*	
P_1	*Area* [a]	*4.7*		*3.9*		*8.7*	*2.8*	*20.1*
	(%)	*(55)*		*(45)*		*(75)*	*(25)*	
	C (mM)	**0.43**		**0.52**		**0.96**	**0.31**	**2.22**
P_2	*Area*	*4.7*		*3.7*		*13.1*	*2.8*	*24.4*
	(%)	*(56)*		*(44)*		*(82)*	*(18)*	
	C (mM)	**0.44**		**0.35**		**1.23**	**0.26**	**2.28**
P_3	*Area*	*4.4*		*3.4*		*13.4*	*3.2*	*24.3*
	(%)	*(56)*		*(44)*		*(81)*	*(19)*	
	C (mM)	**0.40**		**0.31**		**1.22**	**0.29**	**2.23**
P_4		*Isomer 1*	*Isomer 2*	*Isomer 3*	*Isomer 4*	*Isomer max*	*Isomer min*	Sum
	Area	*3.3*	*2.2*	*0.51*	*0.32*	*23.2*	*1.5*	*31.0*
	(%)	*(52)*	*(34)*	*(8)*	*(6)*	*(94)*	*(6)*	
	C (mM)	**0.24**	**0.16**	**0.04**	**0.02**	**1.67**	**0.11**	**2.24**

a) The area values are reported after normalization to 100 on the total mixture.

Table SI-2 : Concentration of every compound obtained from the integration of the signals of the mixture of imines.

		P_xA	**P_xA+**
P_1	*Area*	*13.5*	*11.2*
	C (mM)	**1.22**	**1.00**
P_2	*Area*	*8.8*	*20.7*
	C (mM)	**0.68**	**1.60**
P_3	*Area*	*5.1*	*22.1*
	C (mM)	**0.42**	**1.81**
P_4[a]	**C (mM)**	**0.34**	**1.90**

a) Due to problems with equilibration, the concentrations relative to **P_4** are calculated separately, from the comparison of the relative stabilities of **P_4H/P_4A** and **P_4A-P_4A+** obtained in the competition experiments (see paragraph 5).

Table SI-3: Values of K_{eq} and $\Delta G°$ (kJ.mol^{-1}) obtained from calculated concentrations.

	$P_xH + H^+ \rightarrow P_xH^+ + H$[b] ΔG° kJ/mol (298K) (*$K_{eq}$*)	**$P_xA + A^+ \rightarrow P_xA^+ + A$** ΔG° kJ/mol (298K) (*$K_{eq}$*)
P_1	-0.02 (*1.01*)	-0.2 (*1.08*)
P_2	-0.8 (*1.42*)	-2.8 (*3.10*)
P_3	-1.2 (*1.61*)	-4.3 (*5.68*)
P_4	-2.7 (*2.95*)	-4.3[a]

a) Due to problems with equilibration, the concentrations relative to P_4 are calculated separately, from the comparison of the relative stabilities of **P_4H/P_4A** and **P_4A-P^4A+** obtained in the competition experiments (see paragraph 5).

5. General procedure for the competition experiments between amine A and hydrazide H

The concentration of every stock solution of scaffolds **P_{1-3}** (20-50 mM in CD_3CN) was determined before every experiment using ^{13}C-DMF as external standard. The solutions of **H** (30 mM in CD_3OD) and **A** (580 mM in CD_3OD) were added such to obtain 600 µL of a solution containing either scaffold **P_{1-3}** (around 3 mM), 1 eq of **H** and 10 eq of **A**. The solutions were then heated for 5 days at 300 K and the equilibrium composition was monitored with ^{13}C NMR. The equilibrium constant was determined by the integration of the signals relative to each couple of compounds. For the scaffold **P_4**, due to the small amount of product available, a non labeled molecule was used for this experiment and the concentration was estimated from ^{1}H NMR. The equilibrium constant was determined in the same manner as before.

Table SI-4: Concentration of hydrazones and imines obtained after integration of the ^{13}C NMR spectrum

		P_XH	**P_XA**	**Sum**	**$P_xA + H \rightarrow P_xH + A$** ΔG° kJ/mol ($K_{eq}$)
P_1	Area[a]	81.8	18.2	100	-13.6
	C (mM)	**2.70**	**0.60**	**3.30**	(*244*)
P_2	Area[a]	90.0	10.0	100	-16.8
	C (mM)	**4.05**	**0.45**	**4.50**	(*902*)
P_3	Area[a]	95.6	4.4	100	-21.0
	C (mM)	**4.01**	**0.18**	**4.20**	(*4840*)

a) The area values are normalized to 100.

Table SI-5: Concentration of hydrazones and imines obtained after integration of the ^{1}H NMR spectrum.

	$P_4A + H \rightarrow P_4H + A$		**$P_4A^+ + H \rightarrow P_4H + A^+$**	
	P4H	P4A	P4H	P4A+
Area[a]	93.4	6.6	85.2	14.8
C (mM)	**2.34**	**0.16**	**2.13**	**0.37**
ΔG° kJ/mol (K_{eq})	-19.0 (2150)		-14.7 (382)	

a) The area values are normalized to 100.