Advanced Synthesis & Catalysis

Supporting Information

Supporting Information

Chemo-enzymatic synthesis and inhibitory activities of hyacinthacines A_1 and A_2 stereoisomers

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Experimental section

Materials. Fructose-1,6-diphosphate aldolase from rabbit muscle (RAMA; EC 4.1.2.13, crystallized, lyophilized powder, 19.5 U mg^{-1}) was from Fluka (Buchs, Switzerland). Rhamnulose-1-phosphate aldolase (RhuA; EC 4.1.2.19, suspension 100 U mL^{-1}) was kindly donated by Boehringer Mannhein (Mannhein, Germany). L-Fuculose-1-phosphate aldolase (FucA, EC 4.1.2.17, lyophilized 500-800 U g^{-1} or as $(NH_4)_2SO_4$ suspension (25 U mL^{-1}) was from Departament d'Enginyeria Química of the Universitat Autònoma de Barcelona, produced from a recombinant *E. coli* (ATCC no 86984) and purified by affinity chromatography. Acid phosphatase (PA, EC 3.1.3.2, 5.3 U mg^{-1}) was from Sigma (St. Louis, USA). The precursor of dihydroxyacetone phosphate (DHAP), dihydroxyacetone phosphate dimer bis (ethyl ketal), was synthesized in our lab using a procedure described by Jung *et al.*[1] with slight modifications. Aluminium oxide 90 active neutral was purchased from Merck. Celite-545 (particle size 26 μm, mean pore diameter 17.000 nm, specific surface area BET method 2.19 m^2 g^{-1}) was obtained from Fluka. β-D-Glucosidase from sweet almonds, α-D-glucosidase type V from rice, α-D-mannosidase from jack bean, α-L-rhamnosidase (naranginase) from *Penicillium decumbens* and β-D-galactosidase grade III: crude from bovine liver and *Aspergillus oryzae*, α-L-fucosidase from bovine kidney and the corresponding substrates *p*-nitrophenyl–α-D-glucopyranoside, *p*-nitrophenyl-β-D-glucopyranoside, *p*-nitrophenyl–α-D-mannopyranoside, *p*-nitrophenyl–α-L-rhamnopyranoside, *p*-nitrophenyl–α-L-fucopyranoside and *p*-nitrophenyl–α-D-galactopyranoside were purchased from Sigma. Deionized water was used for preparative HPLC and Milli-Q-grade water for analytical HPLC. All other solvents used were of analytical grade.

NMR spectroscopy: ^{1}H (500.13 MHz) and ^{13}C (125.76 MHz) nuclear magnetic resonance (NMR) spectra were recorded on an AVANCE 500 Bruker spectrometer equipped with a high-sensitive cryogenically cooled triple-resonance TCI probehead for samples dissolved in D_2O and CD_3OD solutions. Full structural and stereochemical characterization of all compounds was performed with the aid of two dimensional COSY, NOESY, HSQC and HMBC experiments as well as NOE data obtained from selective 1D NOESY experiments recorded with a mixing time of 500 ms. The relative orientation of hydroxyl groups was determined from the characteristic downfield/upfield effects observed for all H-1, H-2, H-3, H-7a proton chemical shift, as recently reported for other iminocyclitols.[2, 3] As a general trend, strong downfield effects are observed for the most characteristic H-1, H-2 and H-3 protons when the corresponding vicinal hydroxyl groups are found in a relative *anti* position. ^1H and ^{13}C chemical shifts were calibrated to a DSS external reference.

Specific rotations were measured with a Perkin Elmer Model 341 (Überlingen, Germany) polarimeter.

HPLC analyses: HPLC analyses were performed on a RP-HPLC cartridge, 250 x 4 mm filled with Lichrosphere® 100, RP-18, 5 µm from Merck (Darmstadt, Germany). Samples (50 mg of the emulsion mixture or 50 µL of the reaction in DMF/water 1:4) were withdrawn from the aldol reaction, dissolved in MeOH (1 mL) to stop the reaction and analyzed by HPLC. The solvent system used was: solvent (A): H_2O 0.1 % (v/v) trifluoroacetic acid (TFA) and solvent (B): ACN/H_2O 4/1 0.095 % (v/v) TFA, gradient elution from 30 % to 90 % B over 30 min, flow rate 1 mL min^{-1}, detection 215 nm. Retention factor (*k*') for the products analyzed by HPLC under the above conditions are given below.

Synthesis of (*S*)- or (*R*)-*N*-benzyloxycarbonyl-pyrrolidinemethanal. ((*R*) or (*S*)-*N*-Cbz-prolinal). To a solution of (*S*) or (*R*)-pyrrolidinemethanol ((*S*)- or (*R*)-prolinol) (49.5 mmol, 5.0 g) in dioxane/water 4:1 (100 mL) was added dropwise Cbz-OSu (47.1mmol, 11.7 g) in dioxane/water 4:1 (10-50 mL) at 25°C. After stirring for 24h, the mixture was evaporated to dryness under reduced pressure. The residue was dissolved with ethyl acetate (150 mL) and washed successively with citric acid 5% w/v (3x50 mL), $NaHCO_3$ 10% w/v (3x50 mL) and brine (2x50 mL). After drying over Na_2SO_4, the organic layer was evaporated under reduced pressure affording a white solid of (*R*)-*N*-Cbz-prolinol (10 g, 85%; 98% purity by HPLC, *k*' 6.6) or (*S*)-*N*-Cbz-prolinol (10.5 g, 95%; 98% purity by HPLC, *k*' 6.6). NMR spectrum analysis of the residue showed only one product. Oxidation of (*R*)- or (*S*)-*N*-Cbz-prolinol (21.2 mmol, 5 g) was efficiently carried out by a procedure described by Ocejo et al.[4] The

(*S*)-*N*-Cbz-prolinal (4.7 g, 96%; 97% purity by HPLC, *k*' 7.0) $[\alpha]_D^{20}$ = -44.5 (*c* = 1.1, MeOH) (lit.[5] $[\alpha]_D^{20}$ = -40.8 (MeOH)); (*R*)-*N*-Cbz-prolinal (4.8 g, 98%; 97% purity by HPLC, *k*' 7.0) $[\alpha]_D^{20}$ = +50.8 (*c* = 1.1, MeOH). The ^{1}H and ^{13}C NMR spectra of (*R*)- or (*S*)-*N*-Cbz-prolinol and (*R*)- or (*S*)-*N*-Cbz-prolinal were consistent with those reported in the literature.[5]

Enzymatic aldol condensation

First reactions were conducted at analytical scale in 10 mL test tubes with screw caps, following the procedure described below.

Enzymatic aldol condensations in emulsions. (*S*)- or (*R*)-prolinal (0.38 mmols, 88 mg), the oil (6% w/w, 0.15 g) and the surfactant (4% w/w, 010 g) were mixed with a vortex mixer. Then, the DHAP solution (0.22 mmols, 2.25 mL of a 96.8 mM solution) at pH 6.9, freshly prepared as described by Effenberger et al.,[6] was added dropwise while stirring at 25ºC with a vortex mixer. The final reaction volume was 2.5 mL. Finally the aldolase, RAMA (55 U), RhuA (1.5 U), or FucA (25 U), was added and mixed again. The test tubes were placed on a horizontal shaking bath (100 rpm) at constant temperature (4ºC or 25ºC depending on the experiment). The reactions were followed by HPLC, as indicated above, until the peak of the product reached a maximum.

Enzymatic aldol condensations in mixtures water/dimethylformamide 4:1. (*S*)- or (*R*)-prolinal (0.38 mmols, 88 mg), was dissolved in DMF (0.56 mL). Then, the DHAP solution (0.22 mmols, 2.25 mL of a 96.8 mM solution) at pH 6.9, prepared as described above, was added dropwise while mixing. The total volume was 2.8 mL. The rest of the experimental procedure was identical to that described for the reaction in emulsions.

Synthesis of 6+7 and 8+9.

Reactions at preparative scale were carried out in Erlenmeyer flasks (250 mL) with screw caps using DMF/water 1:4 as reaction medium. (*S*)-Prolinal (16.1 mmol, 3.75 g) was dissolved in DMF (22.5 mL) and cooled to 0°C. Then, the DHAP solution (9.5 mmol, 90 mL, of a solution 105.2 mM) at pH 6.9, freshly prepared as described by Effenberger et al.,[6] was added at 4ºC under vigorous agitation. The final reaction volume was 112.5 mL. Finally, RhuA (225U) was added and mixed again. The reaction was placed on a horizontal shaking bath (100 rpm) at constant temperature (4ºC). The reaction was followed by HPLC until the peak of the product reached a maximum. The enzymatic reactions were stopped by addition of MeOH (115 mL). Then, the methanol was evaporated and the aqueous solution washed with ethyl acetate (2x50 mL) to remove the unreacted *N*-protected aminoaldehyde. The aqueous layer was collected, the remaining ethyl acetate removed under reduced pressure, and lyophilized. This solid was dissolved in plain water (100 mL) and the pH was adjusted to 5.5. To this solution, acid phosphatase (50 mg, 7 U mg^{-1}) was added. The reaction was followed by HPLC (*k*' 2.2). After 20 h the phosphate group was completely hydrolyzed and no starting material was detected. Then, the reaction mixture was filtered through a 0.45 μm cellulose membrane filter. The filtrate was loaded onto a Perkin-Elmer semipreparative 250x25 mm column, filled with C18, 10 μm and eluted with a step gradient of CH_3CN (0 to 48% over 10 min.) in plain water. Pure fractions were pooled and lyophilized obtaining a pale brown solid (1.03 g, 58% overall yield). (*k*'(**6**) 2.2, *k*'(**7**) 2.5, and *k*'(**9**) 2.5).

Removal of Cbz group and reductive amination. Pd/C (200 mg) was added to a solution of the aldol adduct obtained above (1.03 g) in H_2O/EtOH 4:1 (160 mL). The reaction mixture was shaken under hydrogen gas (50 psi) overnight at room temperature. After removal of the catalyst by filtration through neutralized and deactivated aluminum, the pH of the filtrate was adjusted to pH 5.5, the solvent was evaporated under reduced pressure and then lyophilized. The diasteromeric mixture **6**-**7** (514 mg, 77 %) was afforded as a brown solid.

A similar procedure was followed starting with the (*R*)-Cbz-prolinal obtaining the unphosphated aldol adduct (1.12 g, 63% overall yield) and the mixture of diasteromers **8**-**9** as pale brown solid (656 mg, 90%).

Characterization of the products from the lyophilisates was accomplished by NMR spectroscopy.

*Purification by ion exchange chromatography***.** The diasteromeric mixture **8**+**9** (50 mg) was separated by ion exchange chromatography on a FPLC system following a method described by Asano et al.[7] CM-Sepharose CL-6B (Amersham Pharmacia) in NH_4^+ form stationary phase was packed into a glass column (160 x 20 mm) to a final bed volume of 50 mL. The flow rate was 0.9 ml min^{-1}. The CM-Shepharose-NH_4^+ was washed initially with H_2O. Then, an aqueous solution of the crude material at pH 7 was loaded onto the column. Minor coloured impurities were washed away with H_2O (150 mL, 3 bed volumes). The retained compounds **8** and **9** were released with 125 mL and 285 mL of aqueous 0.01 M NH_4OH, respectively. Pure fractions were pooled and lyophilized affording **8** (20 mg) and **9** (26 mg). The operation was repeated until the whole mixture was consumed. The diasteromers **6** and **7** were separated using the same procedure as above but eluting with ammonium acetate 6 mM. The **6**+**7** mixture (200 mg mixture) was loaded onto the column packed with CM-Shepharose-NH_4^+. After washing with plain water, the retained compound **6** was released with 370 mL of eluent affording **6** (45 mg). A pool with a **6**:**7** proportion of ca 4:1 (71 mg) was collected with 490 mL of eluent and finally a pool with a **6**:**7** proportion of ca 2:1 (66 mg) was released with 600 mL of eluent. The later was reloaded to the column and pure **7** (22 mg) was released with 233 mL of eluent and a mixture **6:7** 3:4 (22 mg) with 120 mL. Finally, the later was reloaded again to the column and eluted with ammonium acetate 8 mM. Thus, **6** (3 mg) was released with 372 mL of eluent and **7** (93% purity by NMR) (11 mg) with 564 mL. Characterization of the products from the lyophilisates from 10 mM ammonium hydroxide was accomplished by NMR spectroscopy.

(1*S*, 2*S*, 3*S*, 7a*S*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine (6). $[\alpha]^{20}_{D}$ = -11.0 (c 1.0 in MeOH) (lit.[8] of the enantiomer $[\alpha]^{20}_{D}$ = + 19.9 (c 0.97 in MeOH). ^{1}H NMR (500.13 MHz, *D_2O*) δ ppm 3.79 (A of an AB system, *J* = 12.2, 8.2 Hz, 1H), 3.78 (bs, 1H), 3.74 (t, *J* = 8.0 Hz, 1H), 3.65 (B of an AB system, *J* = 11.8, 6.5 Hz, 1H), 3.20 (m, 1H), 2.94 (bs, 1H), 2.80 (bs, 1H), 2.75 (bs, 1H), 1.96 (m, 1H), 1.88 (m, 1H), 1.79 (m, 1H), 1.76 (m, 1H) ^{13}C NMR (125.76 MHz, *D_2O*) δ ppm 82.1 (C1), 79.0 (C2), 71.4 (C3), 68.3 (C7a), 64.5 (C8), 57.0 (C5), 31.9 (C7), 26.7 (C6).

(1*R*, 2*S*, 3*R*, 7a*S*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine (7). The specific rotation was measured from a lyophilized solid from a 8 mM ammonium acetate solution. $[\alpha]^{20}_{D}$ = -1.0 (c 0.6 in MeOH) ^{1}H NMR (500.13 MHz, *D_2O*) δ ppm 4.10 (dd, *J* = 3.8, 1.5 Hz, 1H), 3.96 (bt, *J* = 1.9 Hz, 1H), 3.90 (A of an AB system, *J* = 11.9, 6.2 Hz, 1H), 3.84 (B of an AB system, *J* = 11.9, 7.7 Hz, 1H), 3.37 (m, 2H), 3.01 (m, 1H), 2.92 (m, 1H), 2.13 (m, 1H), 1.92 (m, 1H), 1.65 (m, 2H). ^{13}C NMR (125.76 MHz, *D_2O*) δ ppm 83.7 (C1), 81.8 (C2), 74.8 (C3), 67.1 (C7a), 59.6 (C8), 51.2 (C5), 31.9 (C7), 28.7 (C6).

(1*S*, 2*S*, 3*S*, 7a*R*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine (8). $[\alpha]^{20}_{D}$ = -20.0º (c 1.0 in MeOH) (lit.[9] of the enantiomer $[\alpha]^{20}_{D}$ = + 17.0 (c 1.5 in MeOH). ^{1}H NMR (500.13 MHz, *D_2O*) δ ppm 4.15 (dd, *J* = 7.7, 6.5 Hz, 1H), 3.92 (m, 1H), 3.90-3.88 (m, 2H), 3.82 (q, *J* = 7.7, 15.6 Hz, 1H), 3.20 (m, 1H), 3.11 (m, 1H), 2.91 (dt, *J* = 10.4, 6.03 Hz, 1H), 2.00 (bs, 1H), 1.91 (bs, 1H), 1.88 (m, 1H), 1.77 (m, 1H). ^{13}C NMR (125.76 MHz, *D_2O)* δ ppm 78.3 (C1), 77.6 (C2), 66.9 (C7a), 66.6 (C3), 60.8 (C8), 50.4 (C5), 27.6 (C6), 26.3 (C7).

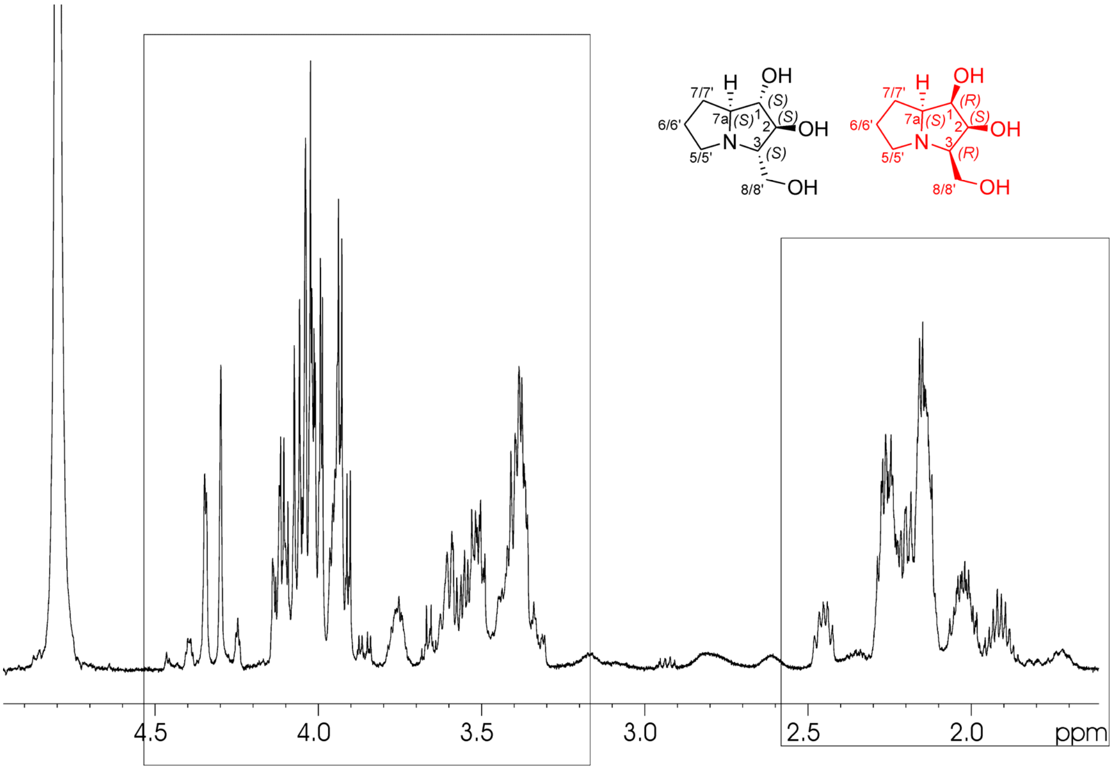
(1*R*, 2*S*, 3*R*, 7a*R*)-1,2-dihydroxy-3-hydroxymethylpyrrolizidine (9). $[\alpha]^{20}_D$ = -34.0º (c 0.9, MeOH) (lit.[10] of the enantiomer $[\alpha]^{20}_D$ = + 3.4 (c 0.32 in H_2O) . ^{1}H NMR (500.13 MHz, *D_2O*) δ ppm 4.31 (dd, *J* = 3.6, 1.6 Hz, 1H), 4.05 (dd, *J* = 4.4, 1.5 Hz, 1H), 3.83 (m, 2H), 3.72 (B of an AB system, *J* = 11.2, 6.5 Hz, 1H), 3.06 (m, 1H), 3.04 (m, 1H), 2.66 (m, 1H), 1.97 (m, 1H), 1.90 (m, 1H), 1.84 (m, 1H), 1.77 (m, 2H). ^{13}C NMR (125.76 MHz, *D_2O*) δ ppm 80.8 (C2), 77.5 (C1), 70.8 (C3), 69.5 (C7a), 61.7 (C8), 56.6 (C5), 29.5 (C6), 25.4 (C7).

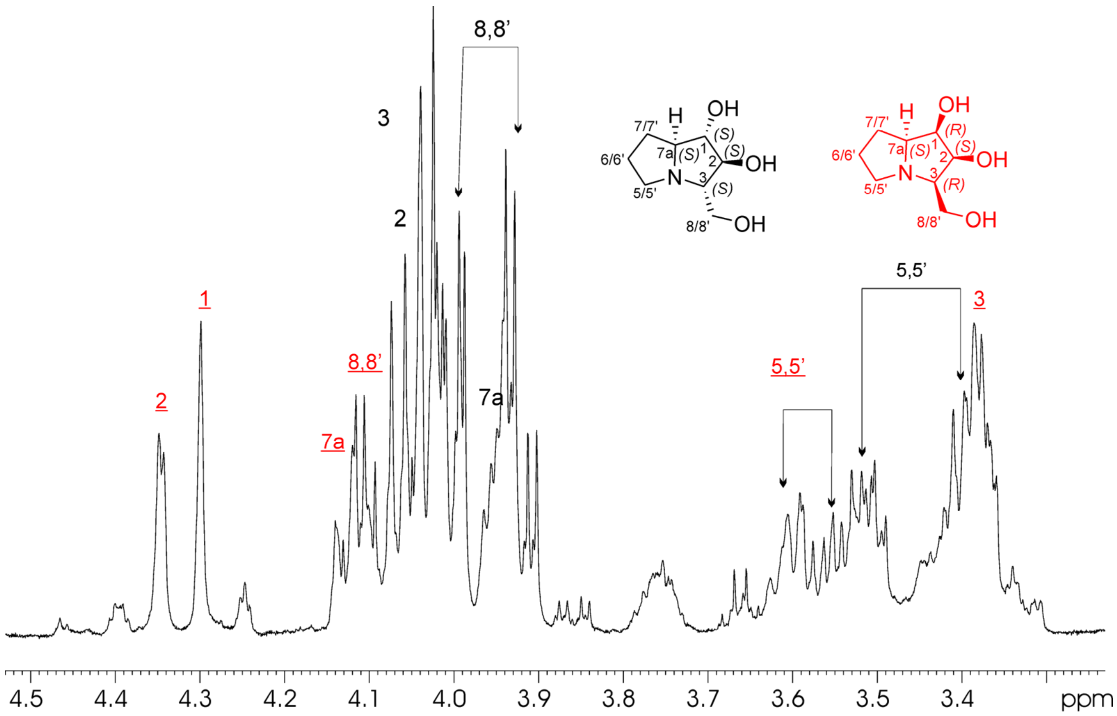
Enzymatic inhibition assays. Commercial glycosidase solutions were prepared with the appropriate buffer and incubated in 96-well plates at 37ºC without (control) or with inhibitor (1.6 mM to 4.2 nM) during 3 min for α-glucosidase, α-D-mannosidase, α-L-rhamnosidase, α-L-fucosidase and 5 min for β-D-galactosidase. After addition of the corresponding substrate solution, incubations were prolonged during different time periods: 10 min for α-D-glucosidase, 3 min for β-D-glucosidase, 6 min for α-D-mannosidase, 5 min for α-L-rhamnosidase, 7 min for α-L-fucosidase and 16 min for β-D-galactosidase and stopped by addition of 50 μL of 1 M Tris solution or 180 μL of 100 mM glycine buffer pH 10, depending on the enzymatic inhibition assay. The amount of *p*-nitrophenol formed was determined at 405 nm with UV/VIS Lector Spectramax Plus (Molecular Devices Corporation) spectrophotometer. β-D-Glucosidase activity was determined with *p*-nitrophenyl-β-D-glucopyranoside (1 mM) in 100 mM sodium acetate buffer (pH 5.0). α-D-Glucosidase from rice activity was determined with *p*-nitrophenyl-α-D-glucopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). α-D-Mannosidase activity was determined with *p*-nitrophenyl-α-D-mannopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). α-L-Rhamnosidase activity was determined with *p*-nitrophenyl-α-D-rhamnopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). α-L-Fucosidase activity was determined with *p*-nitrophenyl-α-D-fucopyranoside (1 mM) in 50 mM sodium acetate buffer (pH 5.0). β-D-Galactosidase activity was determined with *p*-nitrophenyl-β-D-galactopyranoside (1 mM) in 100 mM sodium phosphate buffer, 0.1 mM $MgCl_2$ (pH 7.2). The commercial glycosidase solutions were prepared as follows: β-D-glucosidase: 1 mg/10 mL buffer, α-D-glucosidase: 100 μL commercial suspension/5 mL buffer, α-L-rhamnosidase (naringinase): 0.3 mg/mL buffer; α-D-mannosidase: 25 μL commercial suspension/10 mL buffer; β-D-galactosidase from bovine liver: 0.1 mg /mL buffer, β-D-galactosidase from *Aspergillus oryzae*: 0.5 mg/mL buffer, and α-L-fucosidase 33 μL commercial suspension/10 mL buffer.

Kinetics of Inhibition. The nature of the inhibition against enzymes and the K_i values were determined from the Lineweaver-Burk or Dixon plots.

NMR spectra of the 6+7 and 7+8 mixtures in D_2O: The spectra presented below were recorded from lyophilized aqueous solutions of the mixtures at pH 5.5 without any further purification. The mixtures **6**+**7** and **7**+**8** were obtained as described in methods.

Figure 1. Complete and expanded regions of the ^{1}H NMR spectrum of a mixture of products **6** and **7** obtained after reductive amination of the aldol adduct from the reaction of DHAP with *S*-prolinal catalyzed by RhuA at 25°C and hydrolysis of the phosphate group.

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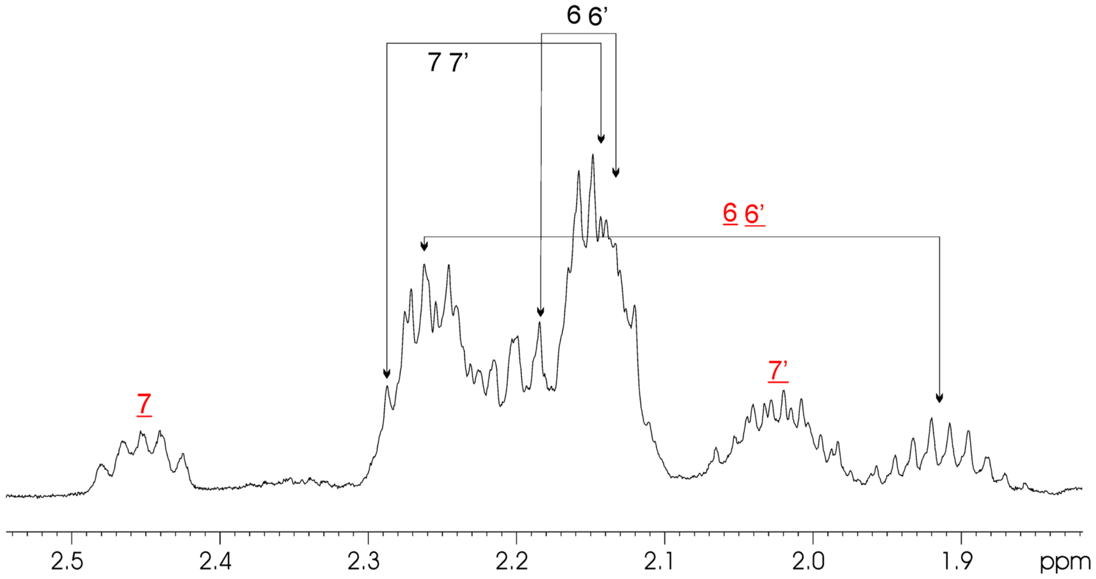
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2.0
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ppm

Figure 2. 2D ^{1}H-^{1}H COSY spectrum of a mixture of products **6** and **7.**

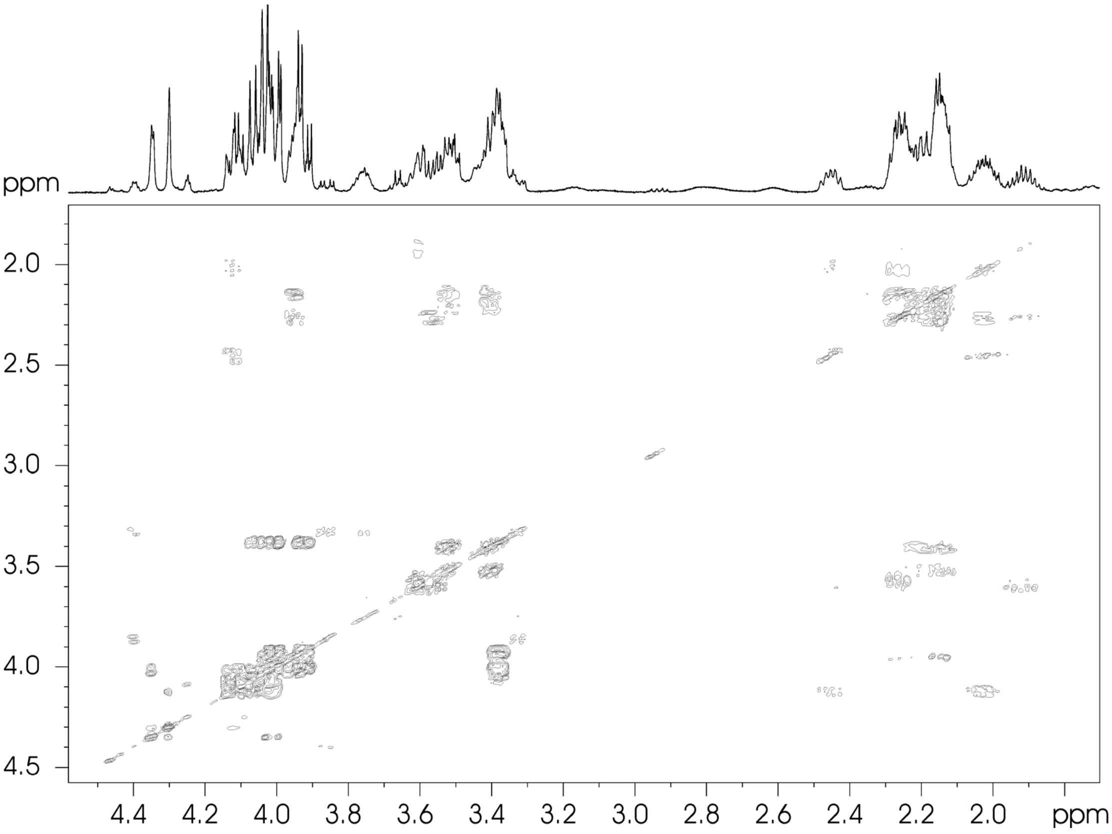

Figure 3. 2D ^{1}H-^{13}C HSQC spectrum of a mixture of products **6** and **7.**

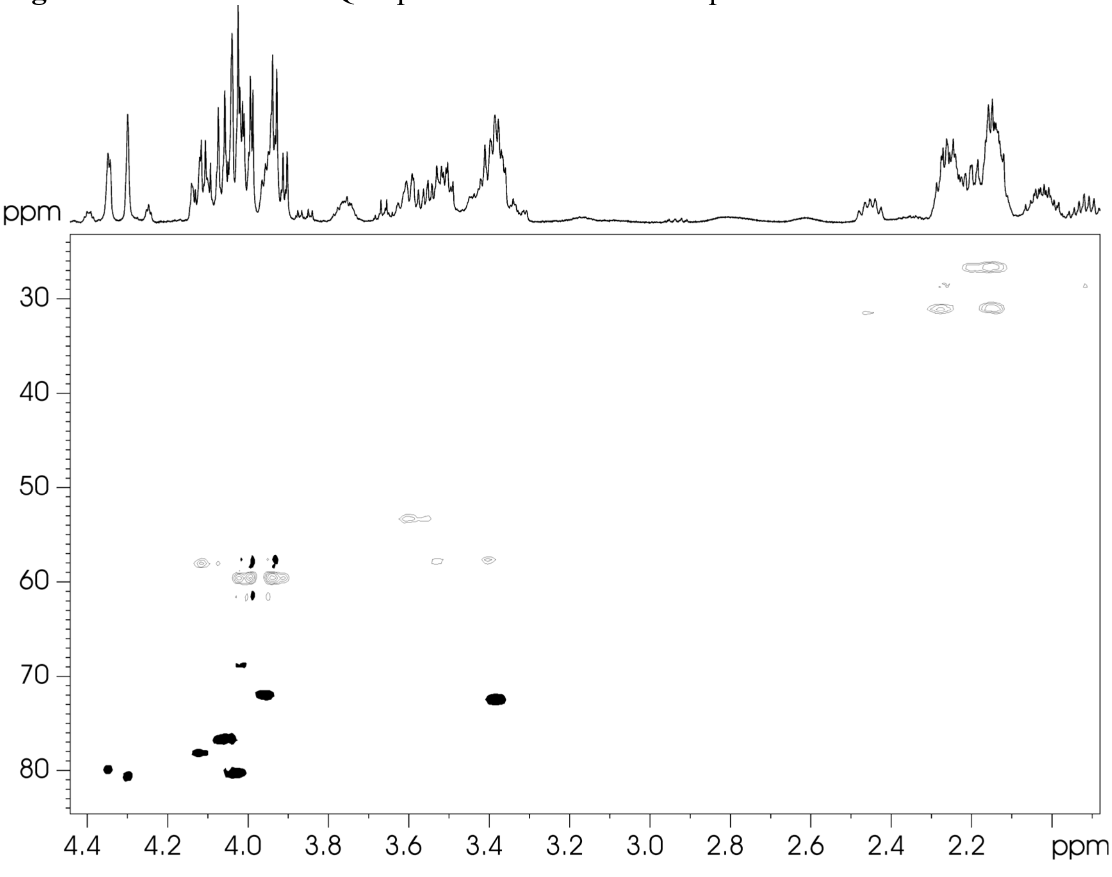

Figure 4. ^{13}C NMR spectrum of a mixture of products **6** and **7.**

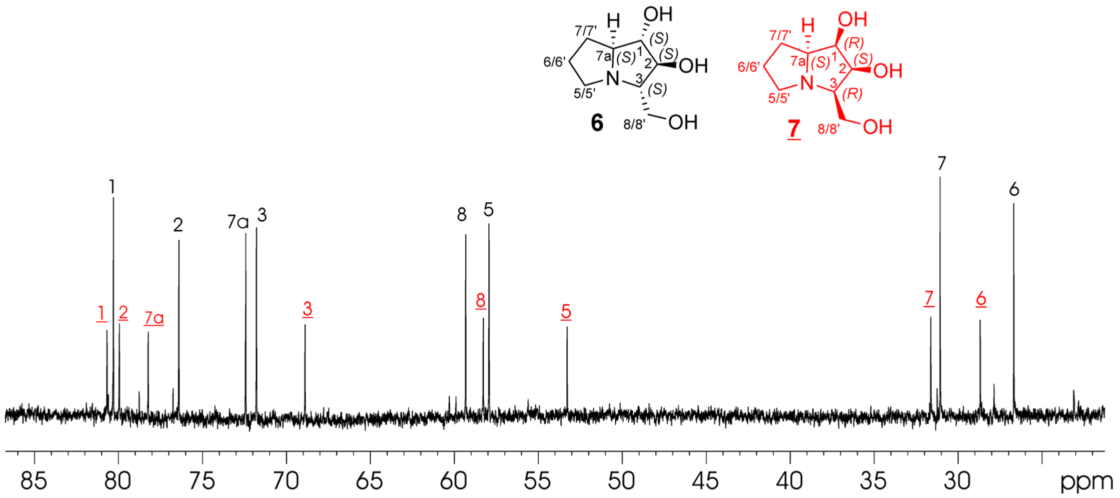

Figure 5. ^{1}H NMR spectrum of a mixture of products **6** and **7** obtained after reductive amination of the aldol adduct from the reaction of DHAP with *S*-prolinal catalyzed by RhuA at 4°C and hydrolysis of the phosphate group. The assignments of the signals are identical as indicated in the enlargements depicted in Figure 1.

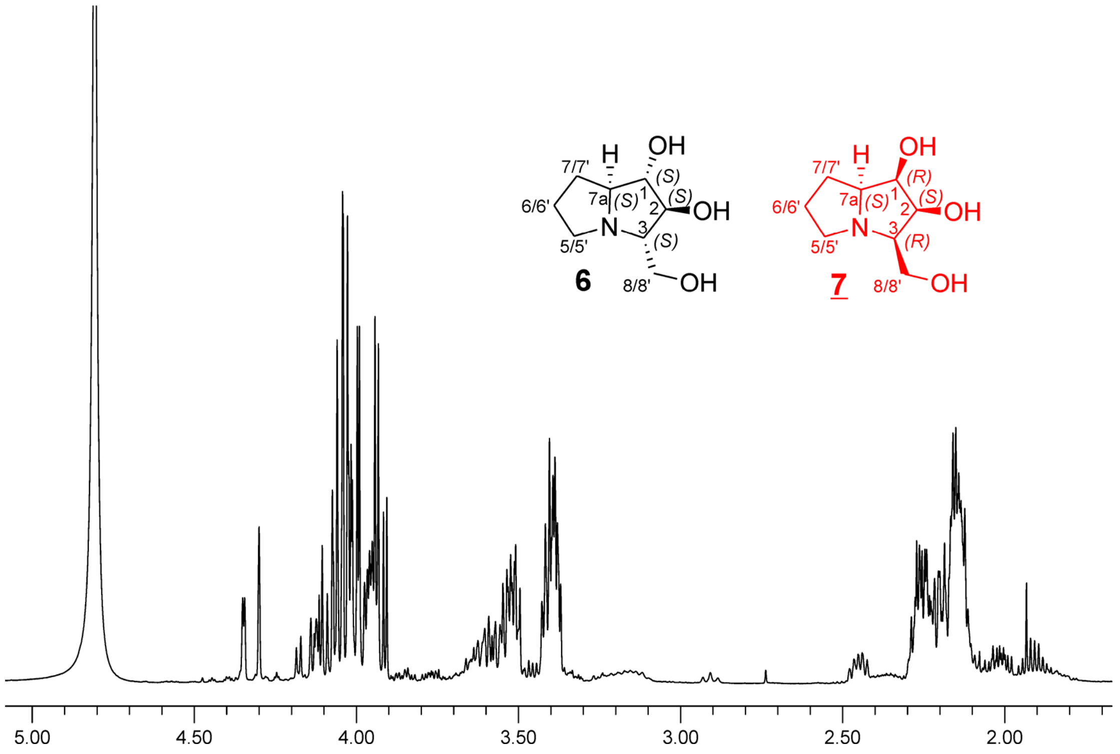

Figure 6. ^{1}HNMR spectrum of a mixture of products **8** and **9** obtained from reductive amination of the aldol adduct from the reaction of DHAP with *R*-prolinal catalyzed by RhuA at 25°C and hydrolysis of the phosphate group.

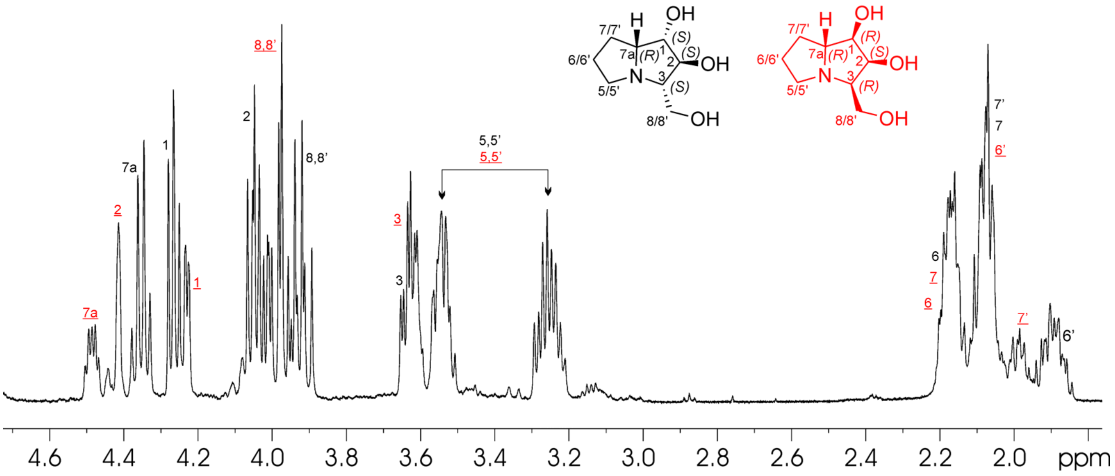

Figure 7. 2D ^{1}H-^{1}H COSY spectrum of a mixture of products **8** and **9.**

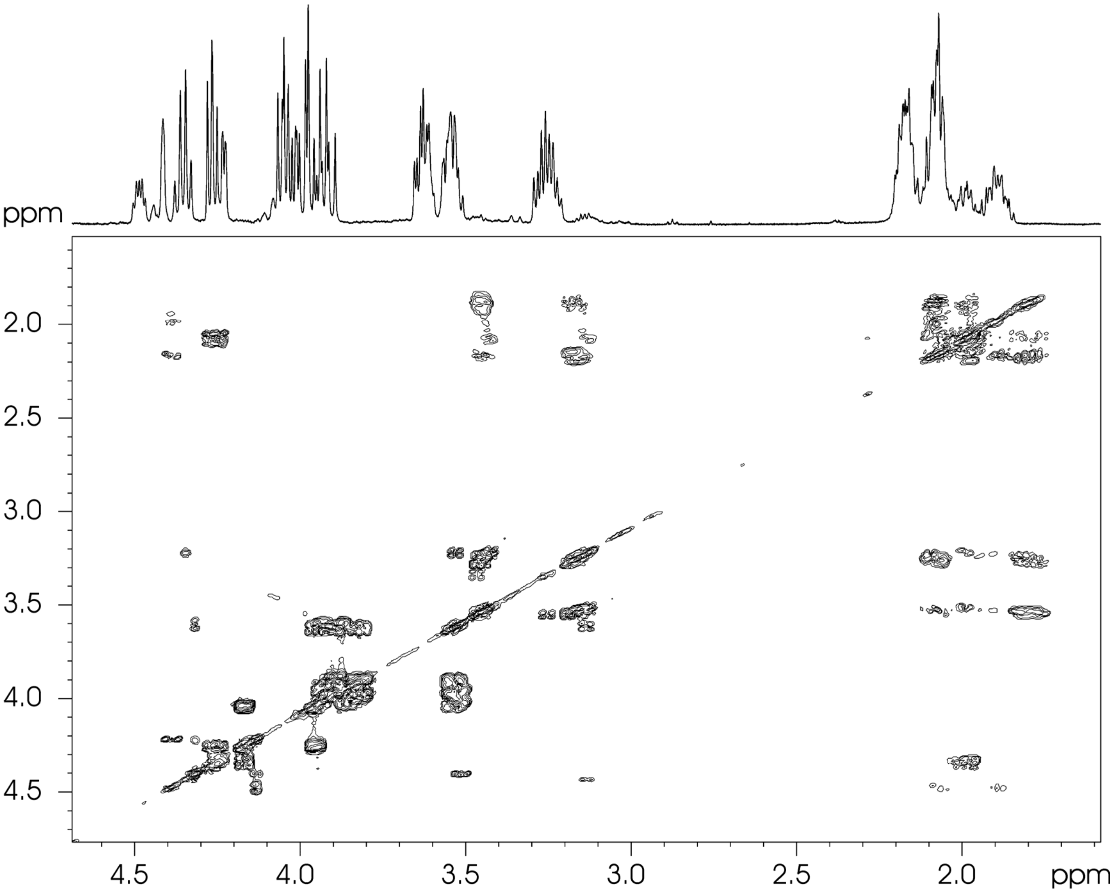

Figure 8. 2D ^{1}H-^{13}C HSQC spectrum of a mixture of products **8** and **9.**

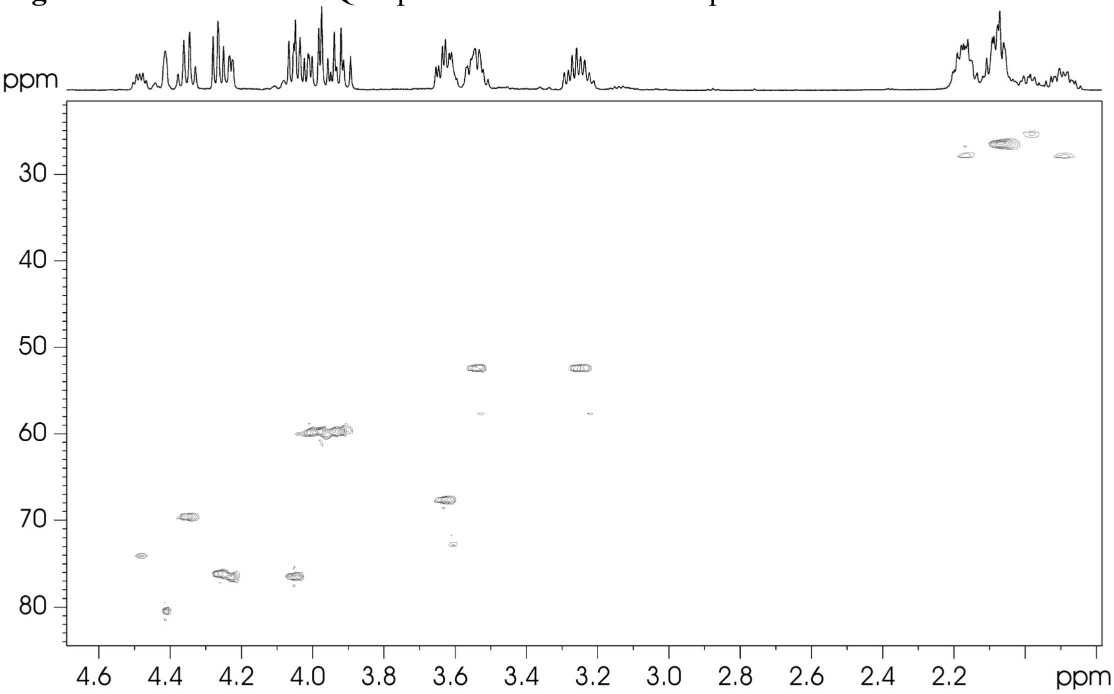

Figure 9. ^{13}C NMR spectrum of a mixture of products **8** and **9.**

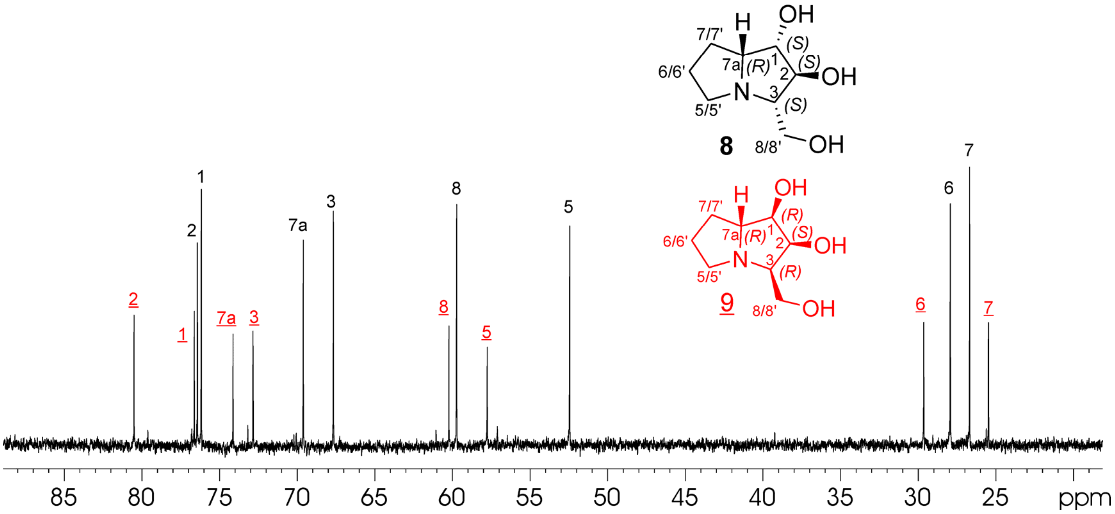

Figure 10. ^{1}H NMR spectrum of a mixture of products **8** and **9** obtained from reductive amination of the aldol adduct from the reaction of DHAP with *R*-prolinal catalyzed by RhuA at 4°C and hydrolysis of the phosphate group.

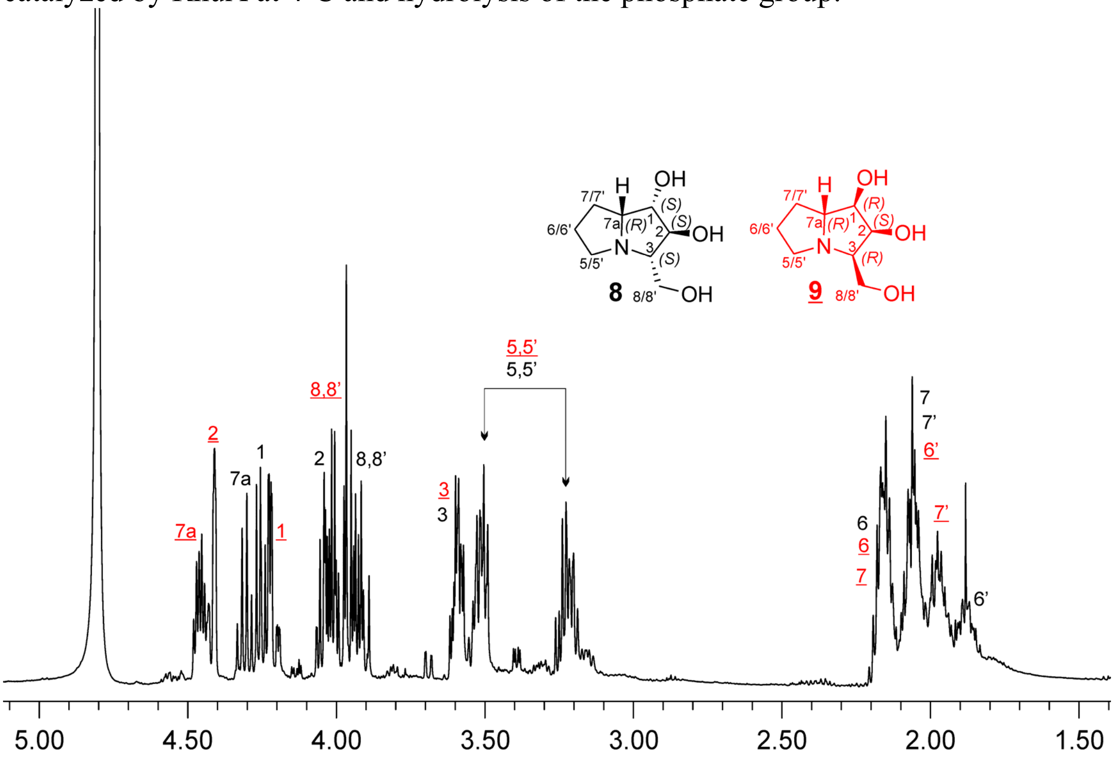

Table 1 Summary of the ^{1}H and ^{13}C chemical shifts for the diasteromeric hyacintacine derivatives **6-9** existing as hydrochloride salt.

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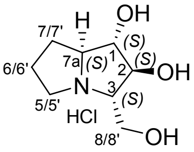
<tr><td colspan="8">
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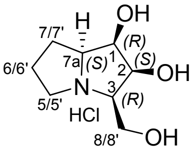
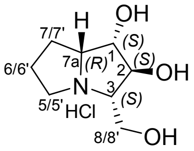
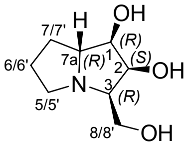
<tr><td>H1</td><td>H2</td><td>H3</td><td>H5/ H5’</td><td>H6/ H6’</td><td>H7a</td><td>H8/ H8’</td><td>H7/ H7’</td></tr>
<tr><td>**4.03**</td><td>**4.06**</td><td>**3.38**</td><td>**3.53**
3.40</td><td>**2.18**
2.13</td><td>**3.94**</td><td>**4.01**
3.93</td><td>**2.28**
2.15</td></tr>
<tr><td>**C1**</td><td>**C2**</td><td>**C3**</td><td>**C5**</td><td>**C6**</td><td>**C7a**</td><td>**C8**</td><td>**C7**</td></tr>
<tr><td>**80.3**</td><td>**76.4**</td><td>**71.8**</td><td>**57.9**</td><td>**26.7**</td><td>**72.4**</td><td>**59.3**</td><td>**31.1**</td></tr>
<tr><td colspan="8">
7</td></tr>
<tr><td>H1</td><td>H2</td><td>H3</td><td>H5/ H5’</td><td>H6/ H6’</td><td>H7a</td><td>H8/ H8’</td><td>H7/ H7’</td></tr>
<tr><td>**4.30**</td><td>**4.35**</td><td>**4.02**</td><td>**3.60**
3.55</td><td>**2.26**
1.92</td><td>**4.12**</td><td>**4.11**
4.08</td><td>**2.45**
2.03</td></tr>
<tr><td>**C1**</td><td>**C2**</td><td>**C3**</td><td>**C5**</td><td>**C6**</td><td>**C7a**</td><td>**C8**</td><td>**C7**</td></tr>
<tr><td>**80.7**</td><td>**80.0**</td><td>**68.9**</td><td>**53.3**</td><td>**28.7**</td><td>**78.2**</td><td>**58.3**</td><td>**31.6**</td></tr>
<tr><td colspan="8">
8</td></tr>
<tr><td>H1</td><td>H2</td><td>H3</td><td>H5/ H5’</td><td>H6/ H6’</td><td>H7a</td><td>H8/ H8’</td><td>H7/ H7’</td></tr>
<tr><td>**4.27**</td><td>**4.05**</td><td>**3.64**</td><td>**3.54**
3.26</td><td>**1.90**
2.16</td><td>**4.35**</td><td>**3.94**
3.91</td><td>**2.08**
2.08</td></tr>
<tr><td>**C1**</td><td>**C2**</td><td>**C3**</td><td>**C5**</td><td>**C6**</td><td>**C7a**</td><td>**C8**</td><td>**C7**</td></tr>
<tr><td>**76.1**</td><td>**76.4**</td><td>**67.7**</td><td>**52.4**</td><td>**27.9**</td><td>**69.6**</td><td>**59.7**</td><td>**26.7**</td></tr>
<tr><td colspan="8">
9</td></tr>
<tr><td>H1</td><td>H2</td><td>H3</td><td>H5/ H5’</td><td>H6/ H6’</td><td>H7a</td><td>H8/ H8’</td><td>H7/ H7’</td></tr>
<tr><td>**4.23**</td><td>**4.41**</td><td>**3.61**</td><td>**3.55**
3.23</td><td>**2.20**
2.06</td><td>**4.48**</td><td>**4.01**
3.95</td><td>**2.20**
1.99</td></tr>
<tr><td>**C1**</td><td>**C2**</td><td>**C3**</td><td>**C5**</td><td>**C6**</td><td>**C7a**</td><td>**C8**</td><td>**C7**</td></tr>
<tr><td>**76.6**</td><td>**80.5**</td><td>**72.8**</td><td>**57.7**</td><td>**29.6**</td><td>**74.1**</td><td>**60.2**</td><td>**25.5**</td></tr>
</table>

Spectra of pure compounds 6, 7, 8 and 9: The mixtures of products **6**+**7**, and **8**+**9** were purified by ion exchange chromatography. Pure compounds were lyophilized from 10 mM ammonium hydroxide solution (basic pH). The spectra presented above were recorded from the corresponding lyophilisate samples in D_2O.

Figure 11. ^{1}H NMR and 2D ^{1}H-^{1}H COSY spectra of **6**.

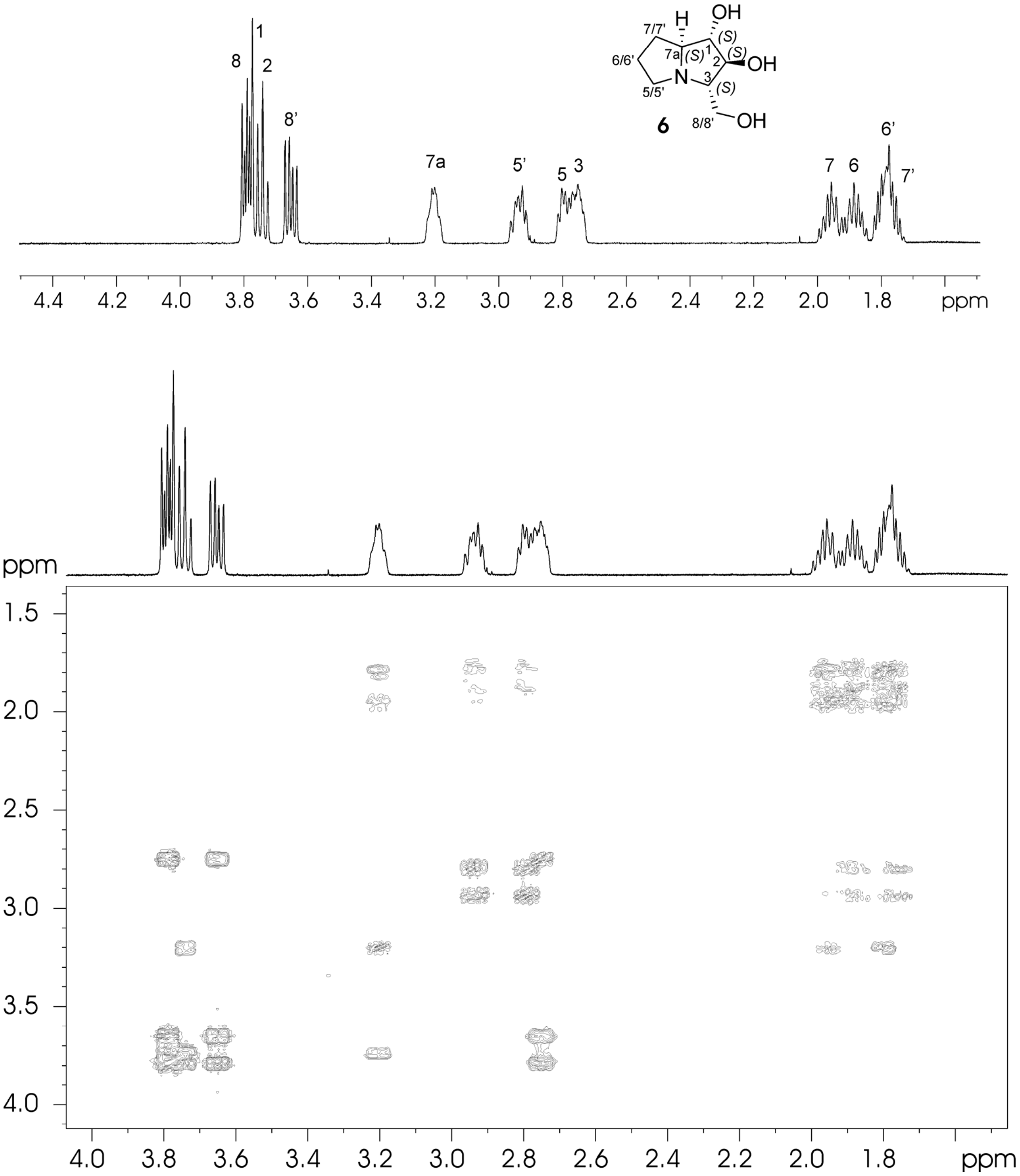


Figure 12. ^{13}C NMR spectrum of **6**.

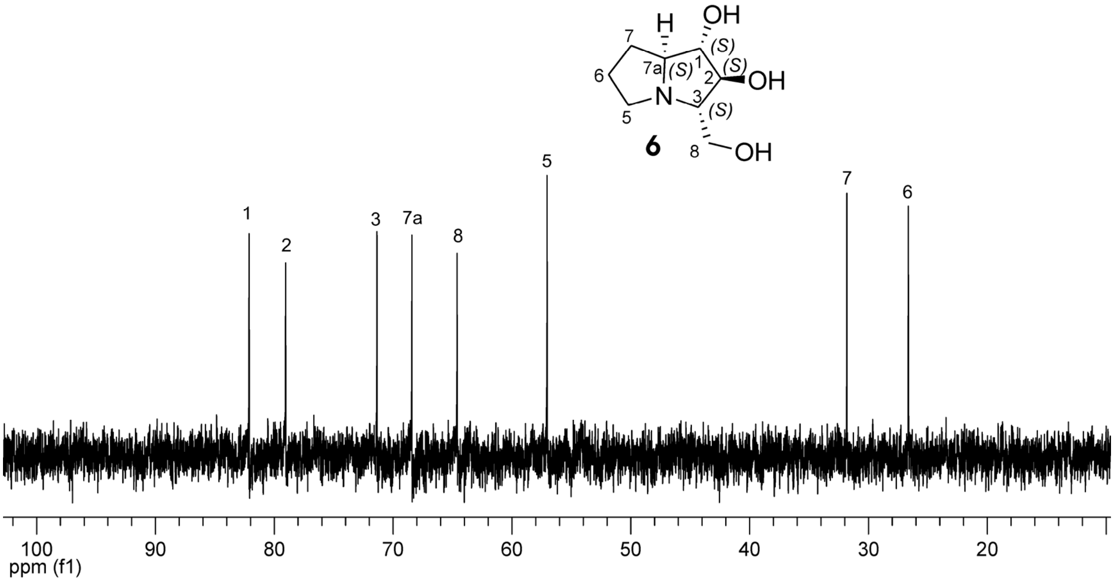


Figure 13. 2D multiplicity-edited HSQC and some selective 1D NOESY spectra of **6**.

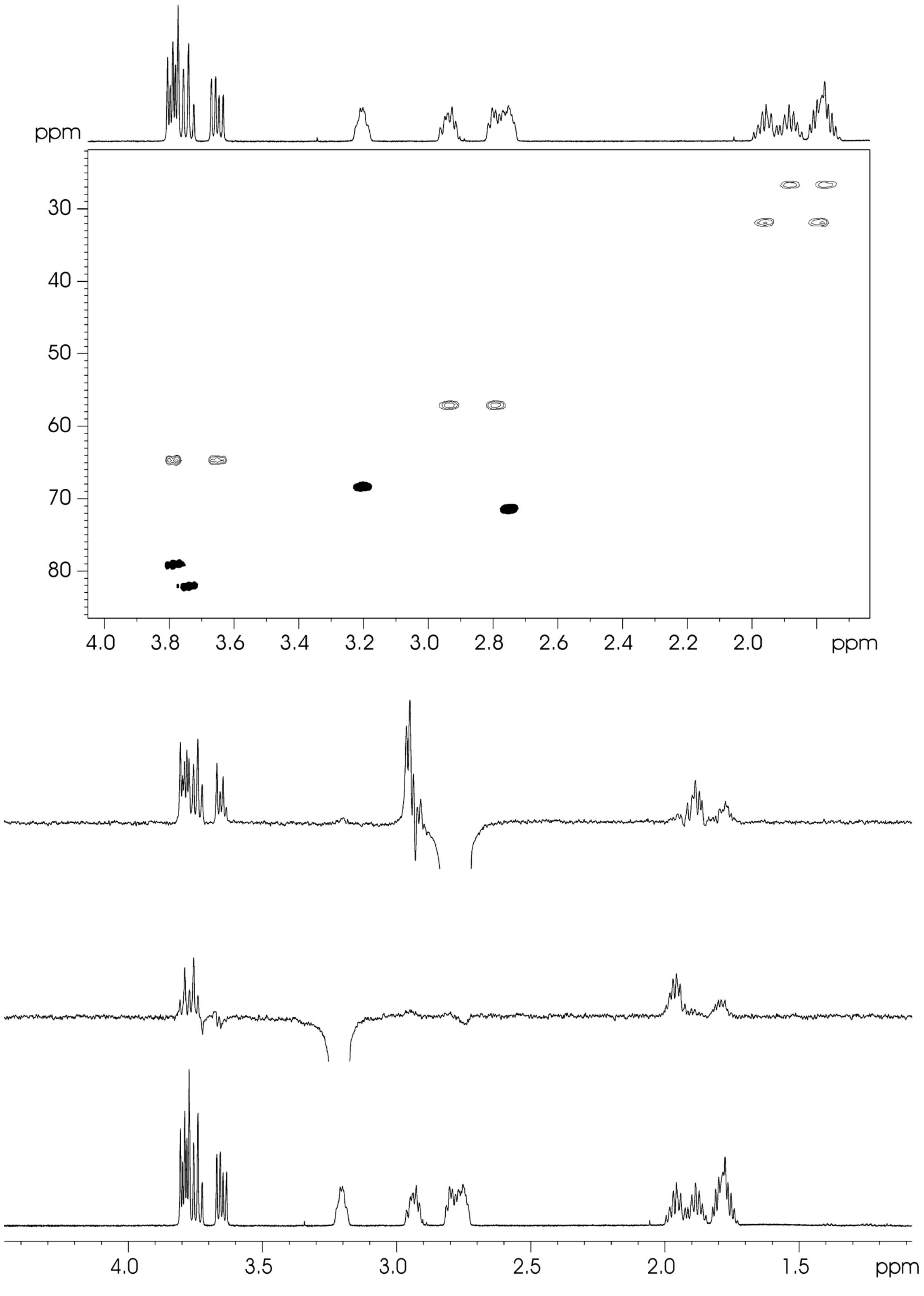


Figure 14. ^{1}H NMR and 2D COSY spectra of **7**.

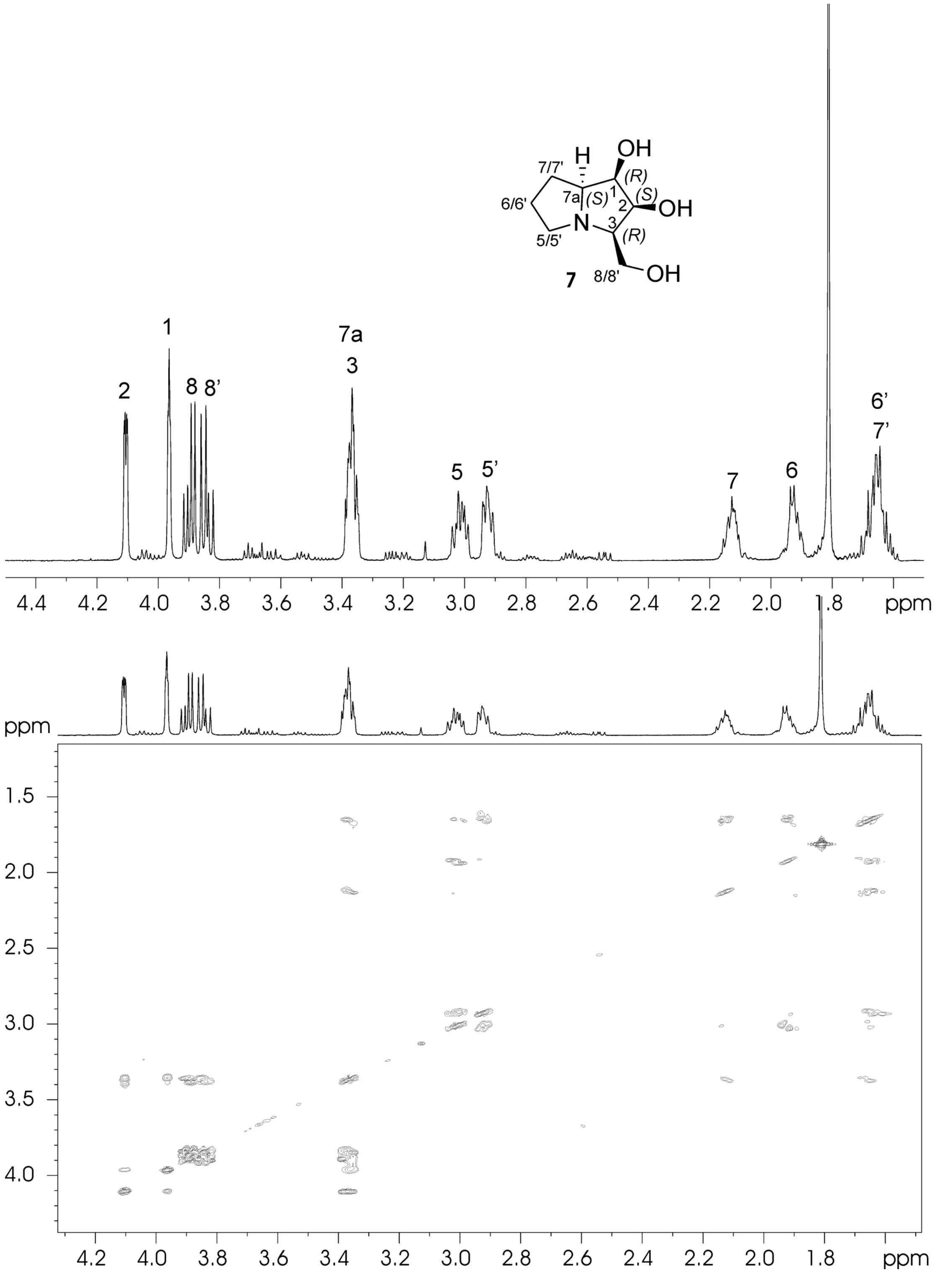


Figure 15. ^{13}C NMR spectrum of **7**. The acetate signal comes from the purification with cation exchange chromatography eluted with ammonium acetate.

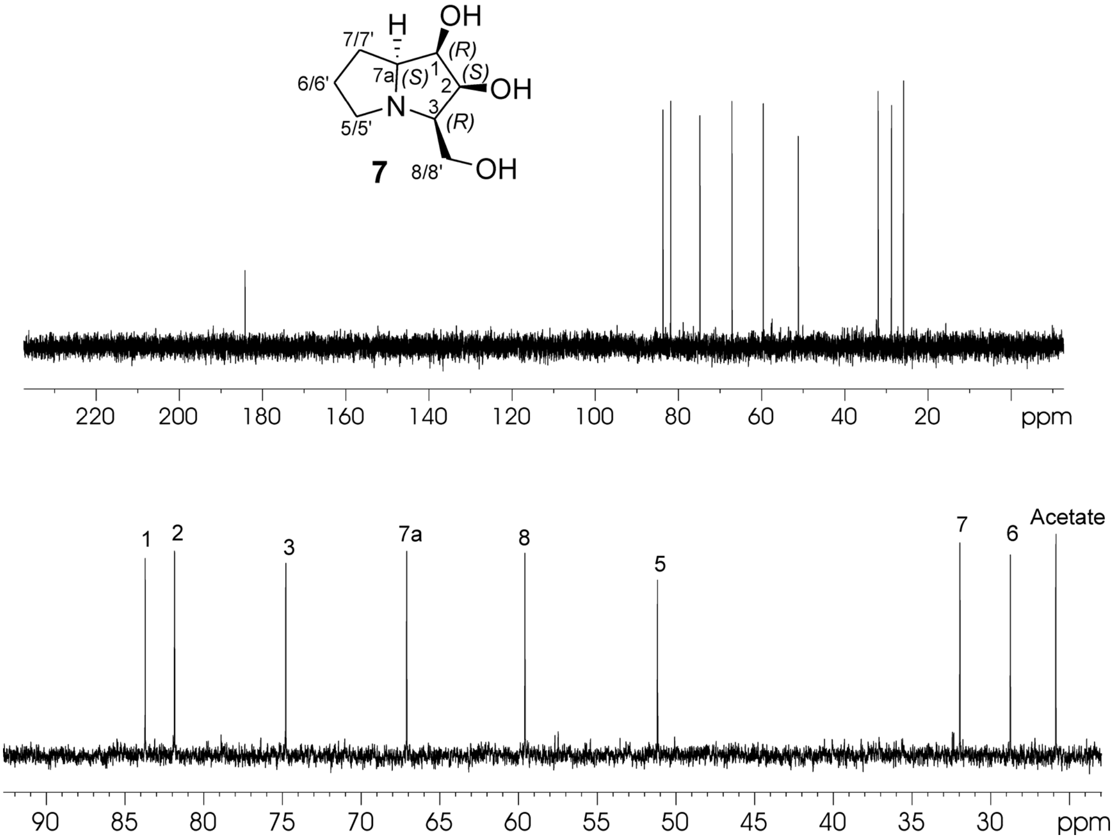


Figure 16. 2D ^{1}H-^{13}C HSQC spectrum of **7**.

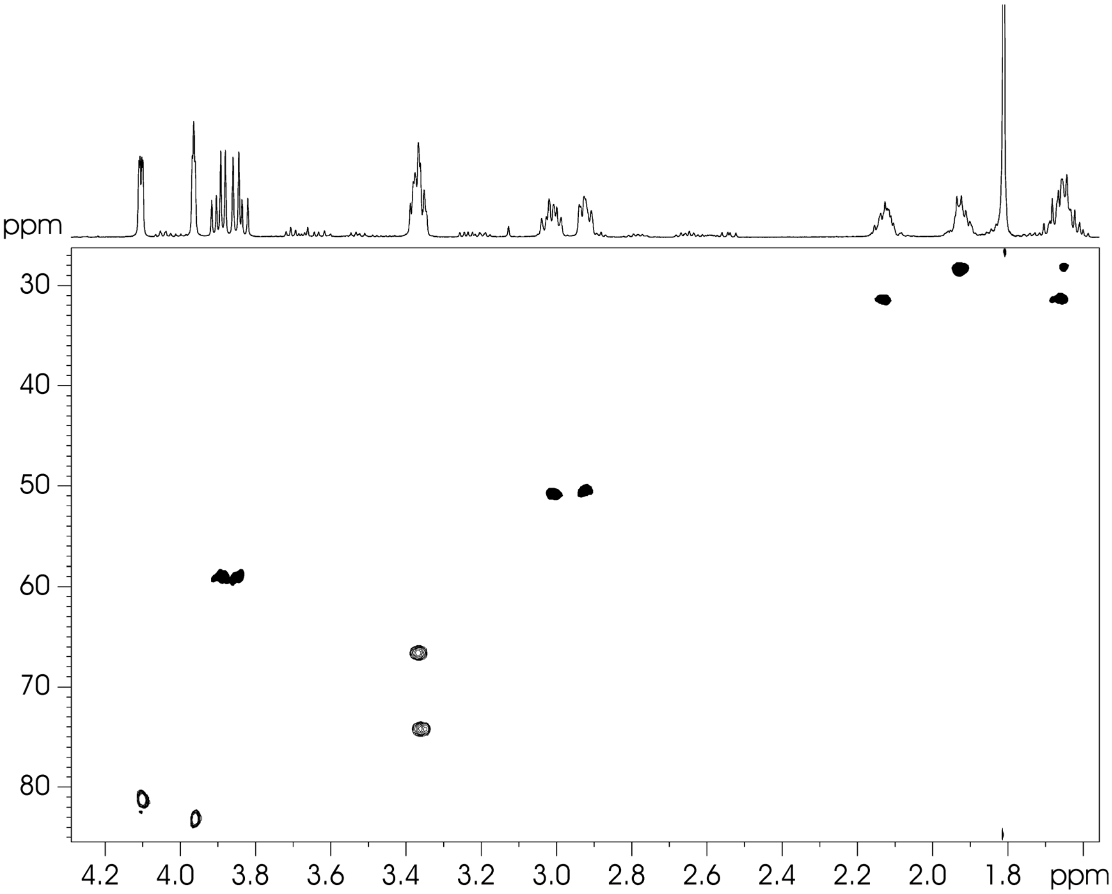


Figure 17. ^{1}H NMR and 2D COSY spectra of **8**.

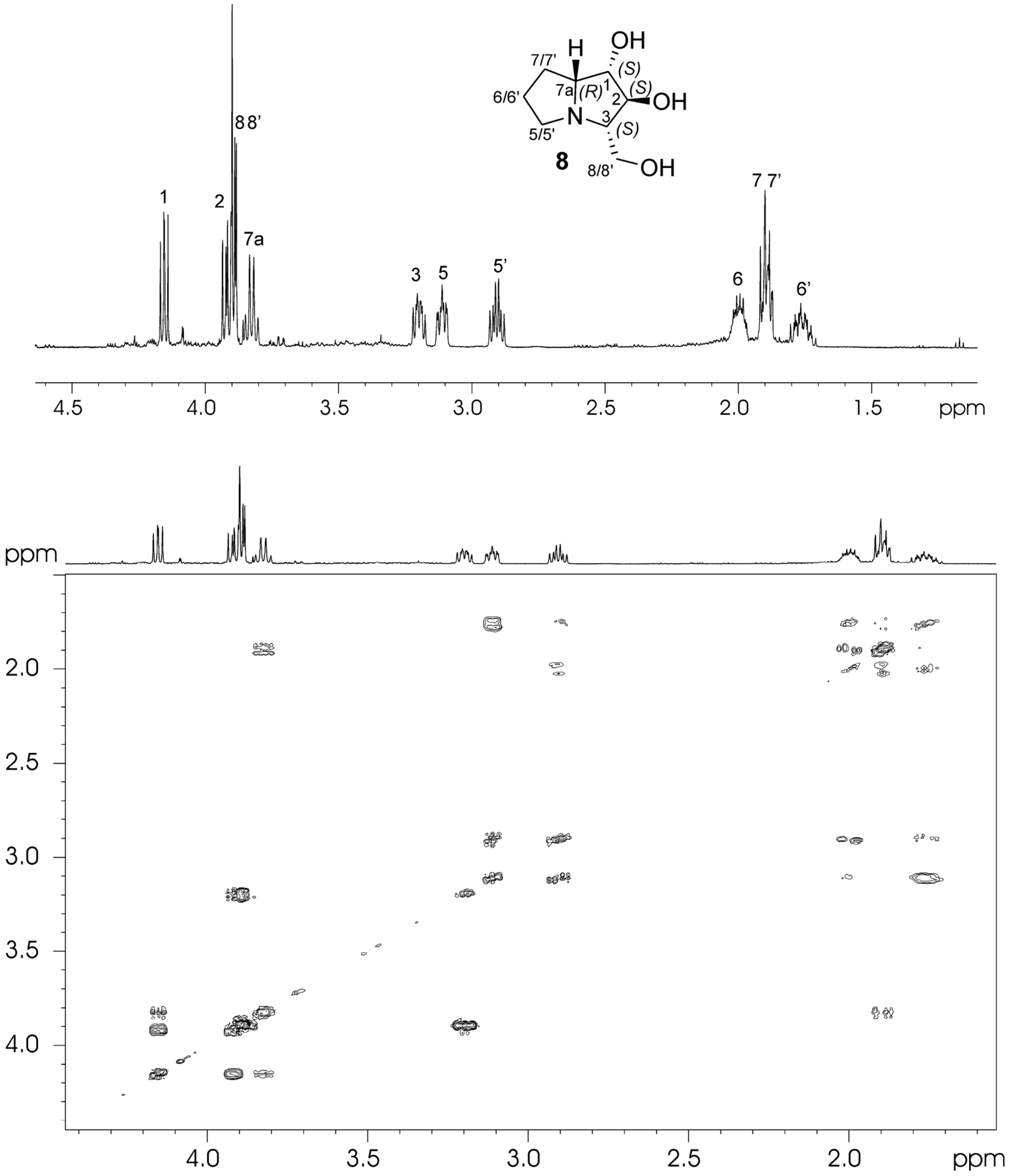


Figure 18. ^{13}C NMR spectrum of **8**.

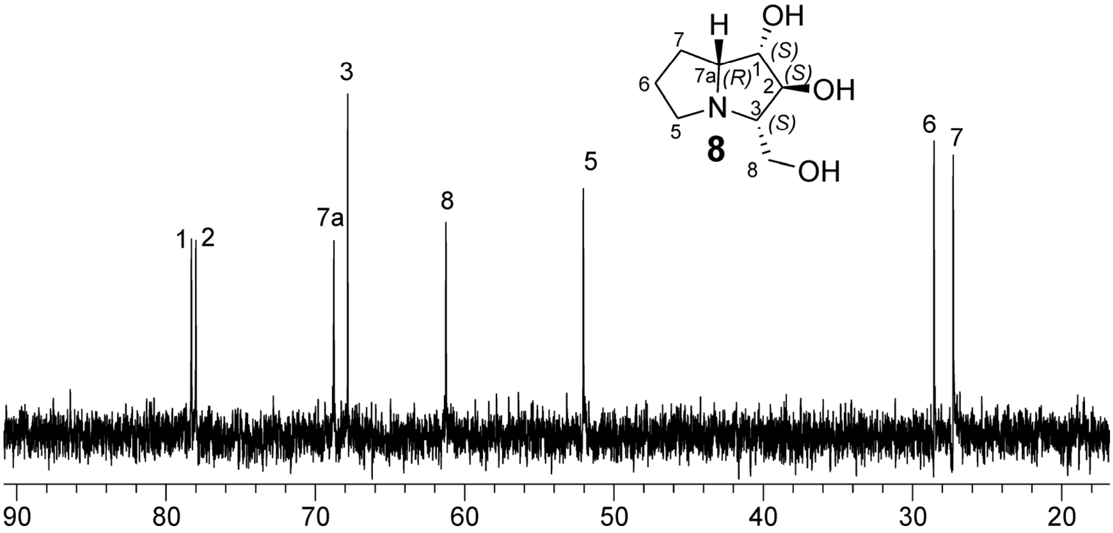


Figure 19. 2D multiplicity-edited HSQC and some selective 1D NOESY spectra of **8**.

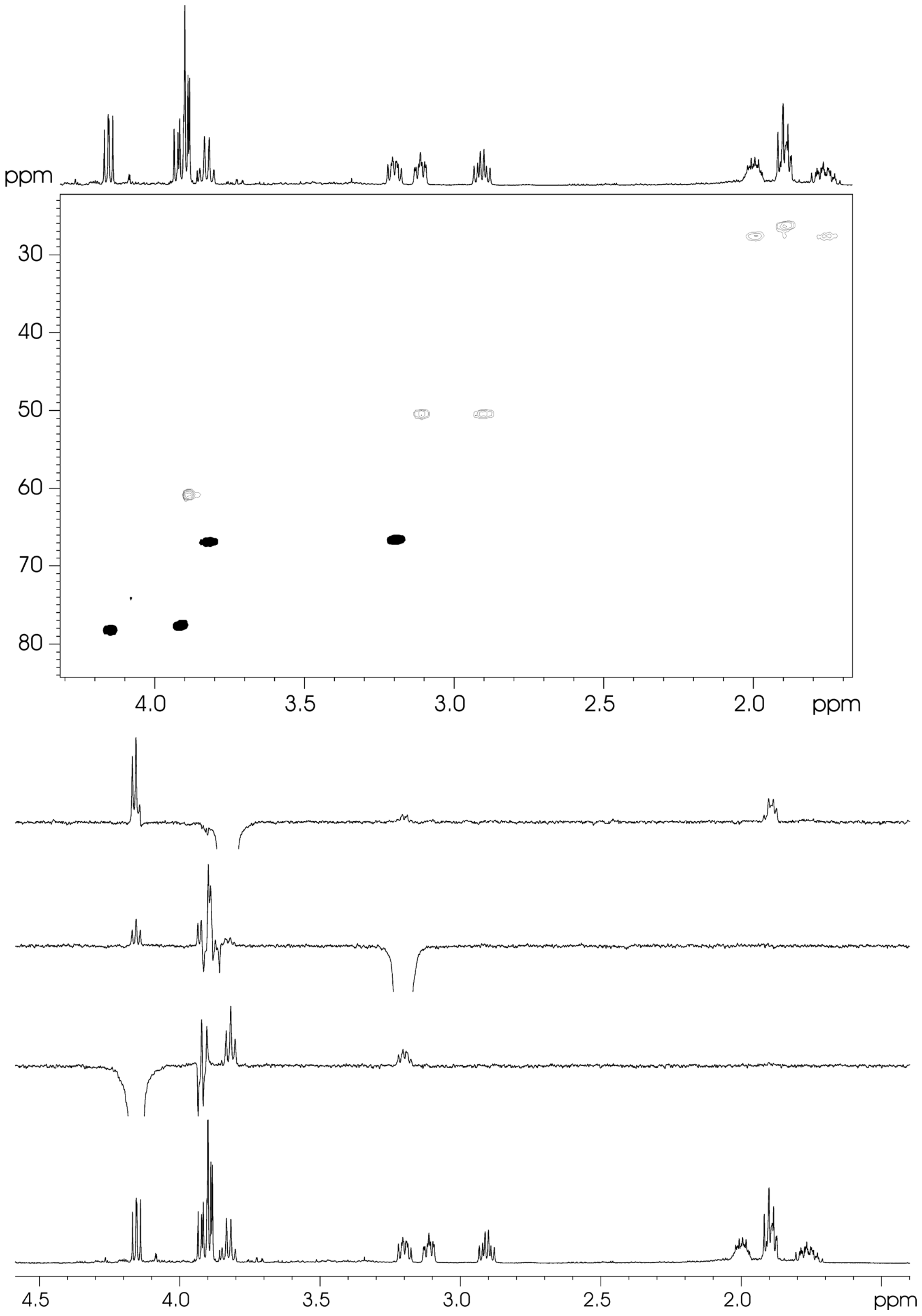


Figure 20. ^{1}H NMR and 2D COSY spectra of **9**.

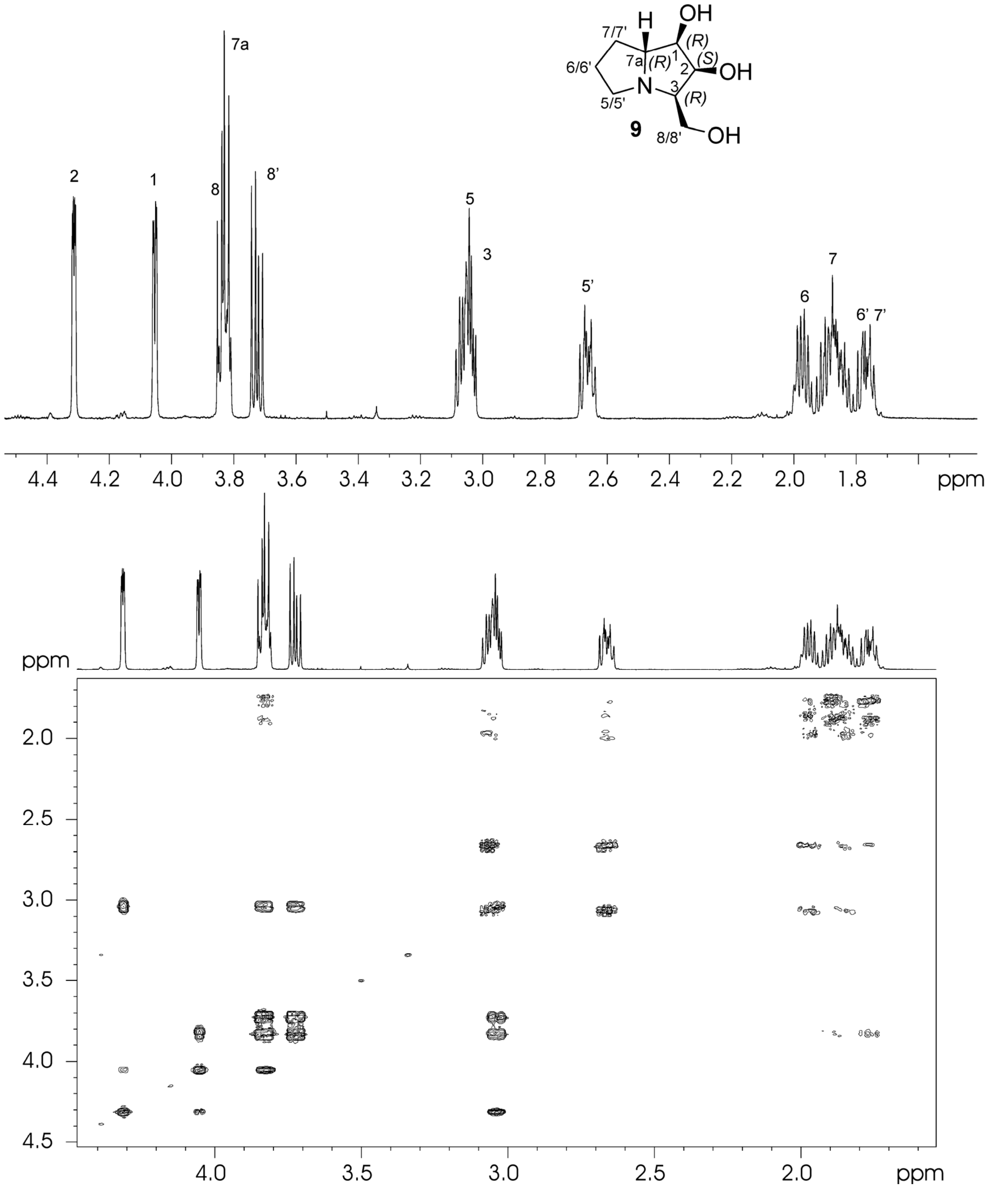


Figure 21. ^{13}C NMR spectrum of **9**.

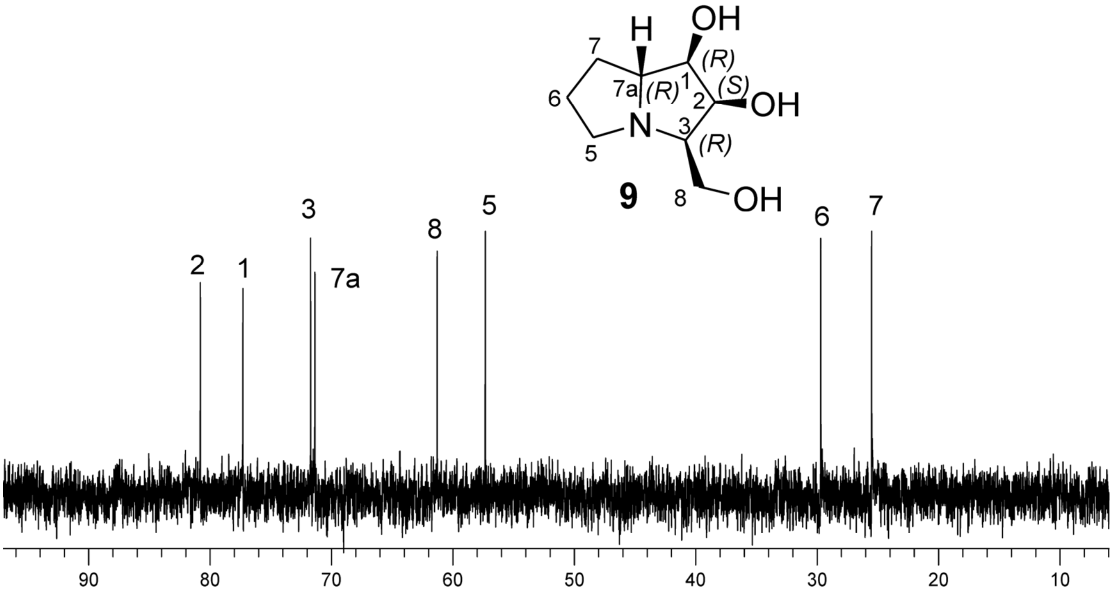


Figure 22. 2D multiplicity-edited HSQC and some selective 1D NOESY spectra of **9**.

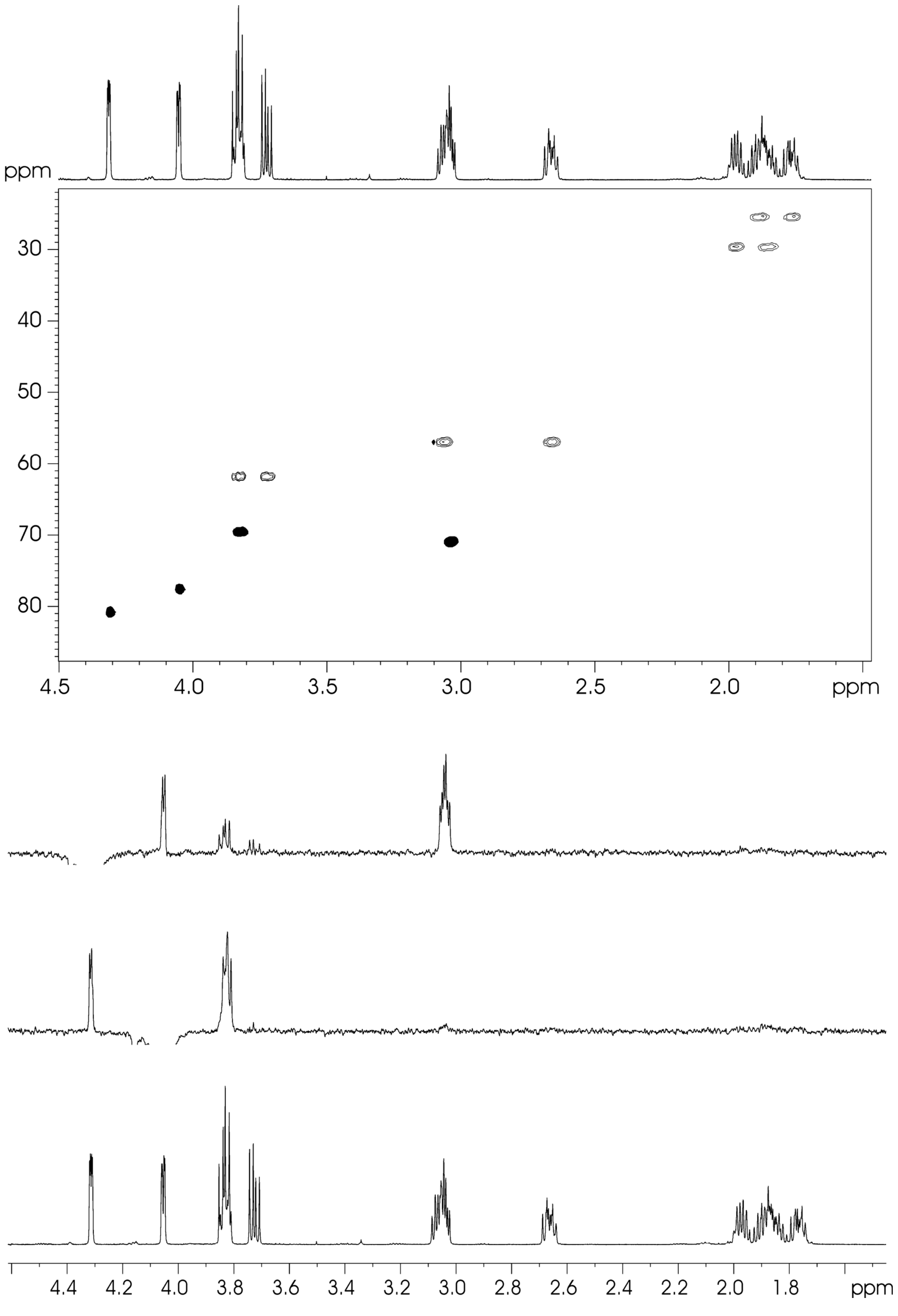
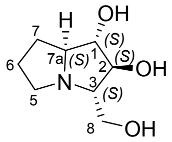
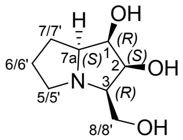


Table 2. Summary of the ^{1}H and ^{13}C chemical shifts of **6-9** as a free base.



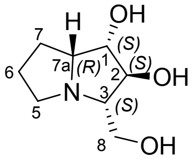
6

H1	H2	H3	H5/ H5’	H6/ H6’	H7a	H8/ H8’	H7/ H7’
3.78	**3.74**	**2.75**	**2.94** **2.80**	**1.88** **1.76**	**3.20**	**3.79** **3.65**	**1.96** **1.79**
C1	**C2**	**C3**	**C5**	**C6**	**C7a**	**C8**	**C7**
82.1	**79.0**	**71.4**	**57.0**	**26.7**	**68.3**	**64.6**	**31.9**



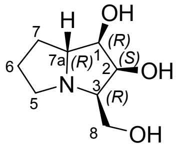
7

H1	H2	H3	H5/ H5’	H6/ H6’	H7a	H8/ H8’	H7/ H7’
3.96	**4.10**	**3.37**	**3.01** **2.92**	**1.92** **1.65**	**3.37**	**3.90** **3.84**	**2.13** **1.65**
C1	**C2**	**C3**	**C5**	**C6**	**C7a**	**C8**	**C7**
83.7	**81.8**	**74.8**	**51.2**	**28.7**	**67.1**	**59.6**	**31.9**



8

H1	H2	H3	H5/ H5’	H6/ H6’	H7a	H8/ H8’	H7/ H7’
4.15	**3.92**	**3.20**	**3.11** **2.91**	**2.00** **1.77**	**3.82**	**3.90** **3.88**	**1.91** **1.88**
C1	**C2**	**C3**	**C5**	**C6**	**C7a**	**C8**	**C7**
78.3	**77.6**	**66.6**	**50.4**	**27.6**	**66.9**	**60.8**	**26.3**



9

H1	H2	H3	H5/ H5’	H6/ H6’	H7a	H8/ H8’	H7/ H7’
4.05	**4.31**	**3.04**	**3.06** **2.66**	**1.97** **1.84**	**3.83**	**3.83** **3.72**	**1.90** **1.77**
C1	**C2**	**C3**	**C5**	**C6**	**C7a**	**C8**	**C7**
77.5	**80.8**	**70.8**	**56.9**	**29.5**	**69.5**	**61.7**	**25.4**

Figure 23. Illustrative example showing the lyophilisation buffer dependence on the ^{1}H NMR spectrum of compound **9**: a) lyophilisation from ammonium hydroxide, b) lyophilisation with ammonium chloride c) ^{1}H NMR recorded after mixture of a) and b).

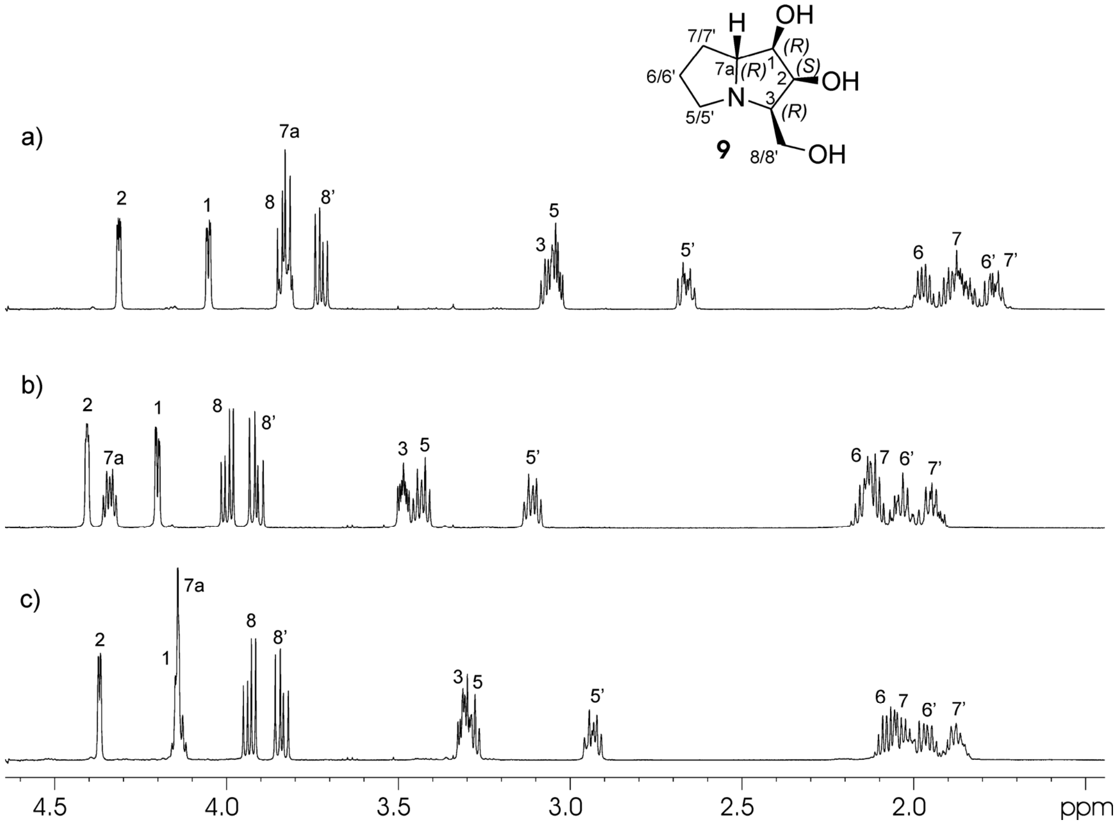
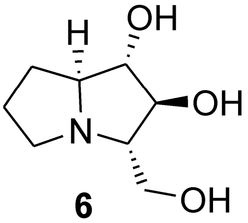


Figure 24. Reciprocal Dixon plot for the determination of inhibition constant (K_i) of compound **6** against α-D-glucosidase from rice.



6

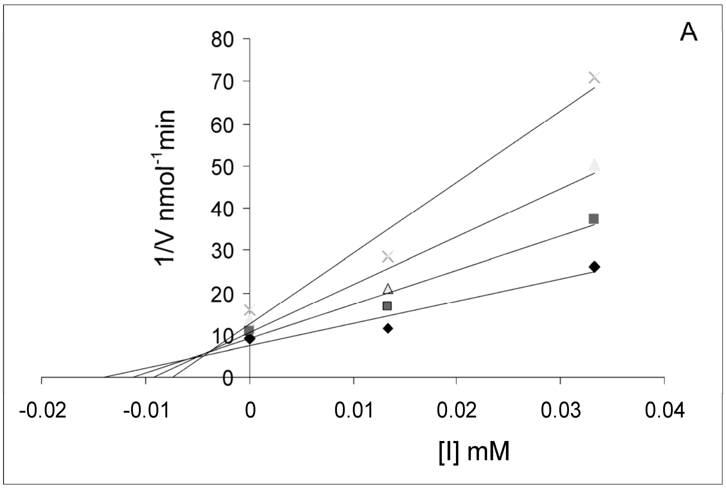
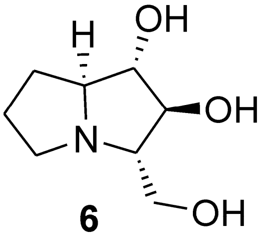


Figure 25. Reciprocal Dixon plot for the determination of inhibition constant (K_i) of compound **6** against α-L-Rhamnosidase *Penicillium decumbens*.



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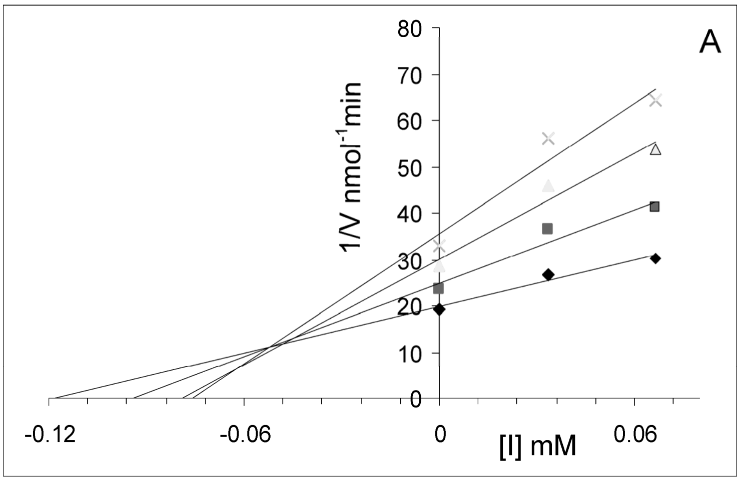
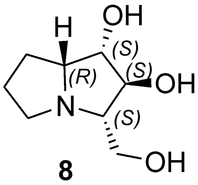
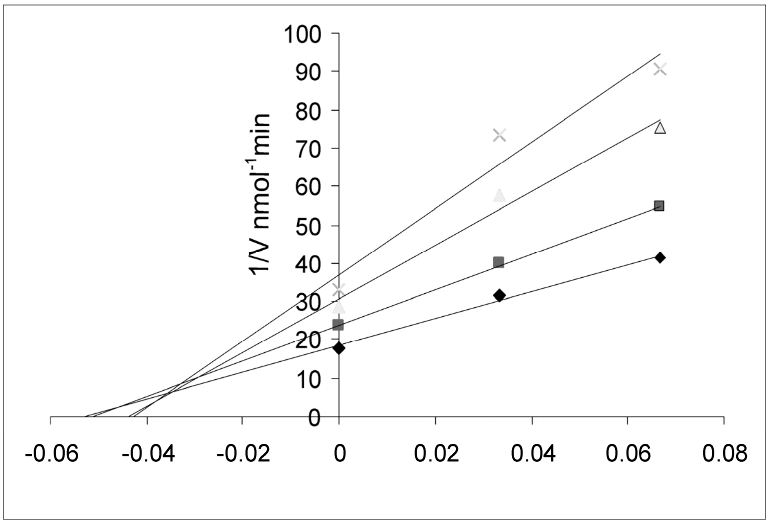


Figure 26. Lineaweaver-Burke double reciprocal plots (A) and reciprocal Dixon plots (B) for the determination of inhibition constant (K_i) of compound **8** against α-L-Rhamnosidase *Penicillium decumbens*.



8



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