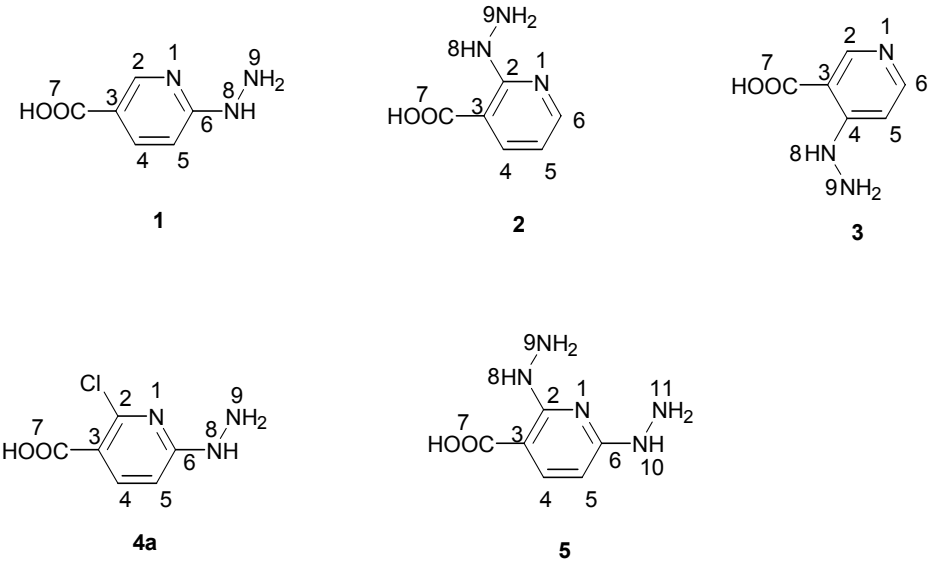
Synthesis and evaluation of analogues of HYNIC as bifunctional chelators for technetium

Levente K Meszaros, Anica Dose, Stefano C G Biagini and Philip J Blower

Electronic Supplementary Information

Content

1. Numbering system for NMR assignments



1 **2** **3** **4a** **5**

Figure E1: NMR assignments for 1-5

2. NMR assignments for 4a

In the ^{13}C NMR spectra, the chemical shift of carbon-3 of **4a** (113.5 ppm), is similar to the carbon-3 chemical shift of compound **1** (115 ppm), indicative of the hydrazine group *para*- to the carboxylic acid group. By contrast, in compound **2** the carboxylic acid group is in an

ortho-orientation with respect to the hydrazine group, and in this case carbon-3 has a chemical shift of 109 ppm. Comparing nicotinic acid with compounds **1**, **2** and **5**, shows that the hydrazine functionality has an appreciable shielding effect on the carbon-3, whereas the influence of the chlorine on carbon-3 chemical shift appears to be minimal, as can be seen by comparing nicotinic acid with **7**, **8** and **9**, in all of which carbon-3 has a similar chemical shift around 127 ppm. A summary is given on Fig. E2.

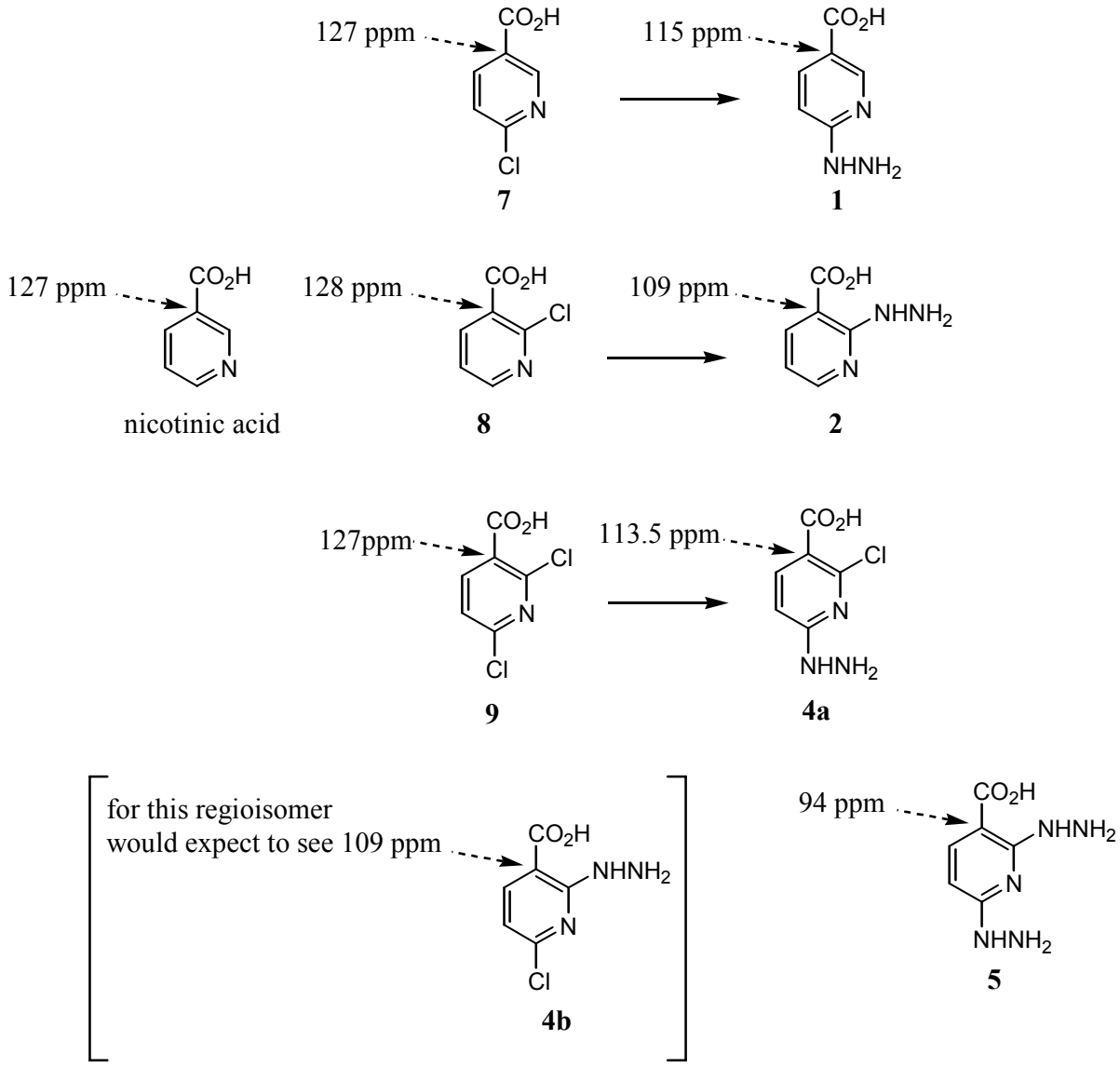


Fig. E2: NMR assignments for 4 and its isomers

3. Optimising the conditions for synthesising 5

The experiments we carried out to set up the optimial conditions for making **5** are summarised in Table E1. Mixtures were analysed by LC-MS (method 1).

Experiment	T (°C)	t (min)	Samples (t (min))
1	100	270	270
2	100	60	0, 20, 40, 60
3	50	1020	1020
4	60	900	240, 900

Table E1: Experiments to determine the optimal conditions to synthesise 5

Experiment 1:

Results

NMR data, recorded on a Bruker 400MHz (NMR assignments are shown on Figure E3):

NMR: δ_H(400MHz; $(CD_3)_2SO$; Me_4Si): 5.92 (2H, d, *J*=8.4, 3-H, 5-H), 5.90 (4H, br, 8-H, 10-H), 6.93 (2H, br, 7-H, 9-H), 7.18 (1H, t, *J*=8.0, 4-H). δ_C(100MHz; $(CD_3)_2SO$; Me_4Si): 94.20 (C-3, C-5), 138.02 (C-4), 160.81 (C-2, C-6). MS: m/z= 140.0928 ($[M+H]^+$, 100%), 123.0663 ($[M-NH_3+H]^+$, 50), 110.0712 ($[M-N_2H_3+H]^+$, 55). MH requires 140.0936.

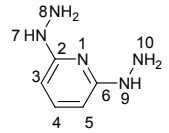
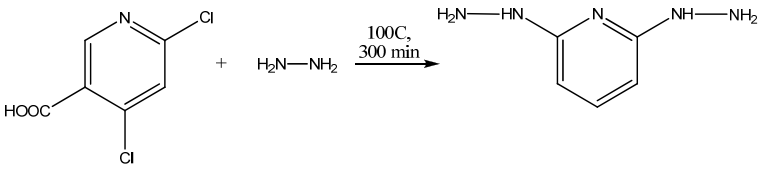


Figure E3: NMR assignments for 2,6-dihydrazinopyridine

Discussion

Based on the LC-MS and NMR data we obtained the applied conditions had resulted in a ~100% conversion to 2,6-dihydrazinopyridine (see Scheme E1):



Scheme E1: The reaction of 9 with hydrazine gives 2,6-dihydrazinopyridine at 100°C

Note that we were unable to detect **5** in the mixture by mass spectrometry. Thus the above conditions are not applicable for synthesising **5**, but this type of reaction might be of use in organic syntheses regarding the ~100% conversion to2,6-dihydrazinopyridine

Experiment 2

In this experiment, the mixture containing the reactants was heated to 95-100°C gradually, with an average speed of 8°C/min. t=0 min refers to the time point when 95°C had been reached.

Results

LC data are shown in Table E2.

Peak	RT (min)	Area (%)			
		t=0	t=20	t=40	t=60
1	0.3	0	100	100	100
2	0.37	100	72.7	0	0

Table E2: LC data for Experiment 2

Note that due to the small difference in retention times compounds reached the mass spectrometer at the same time. MS data are shown below in Table E3.

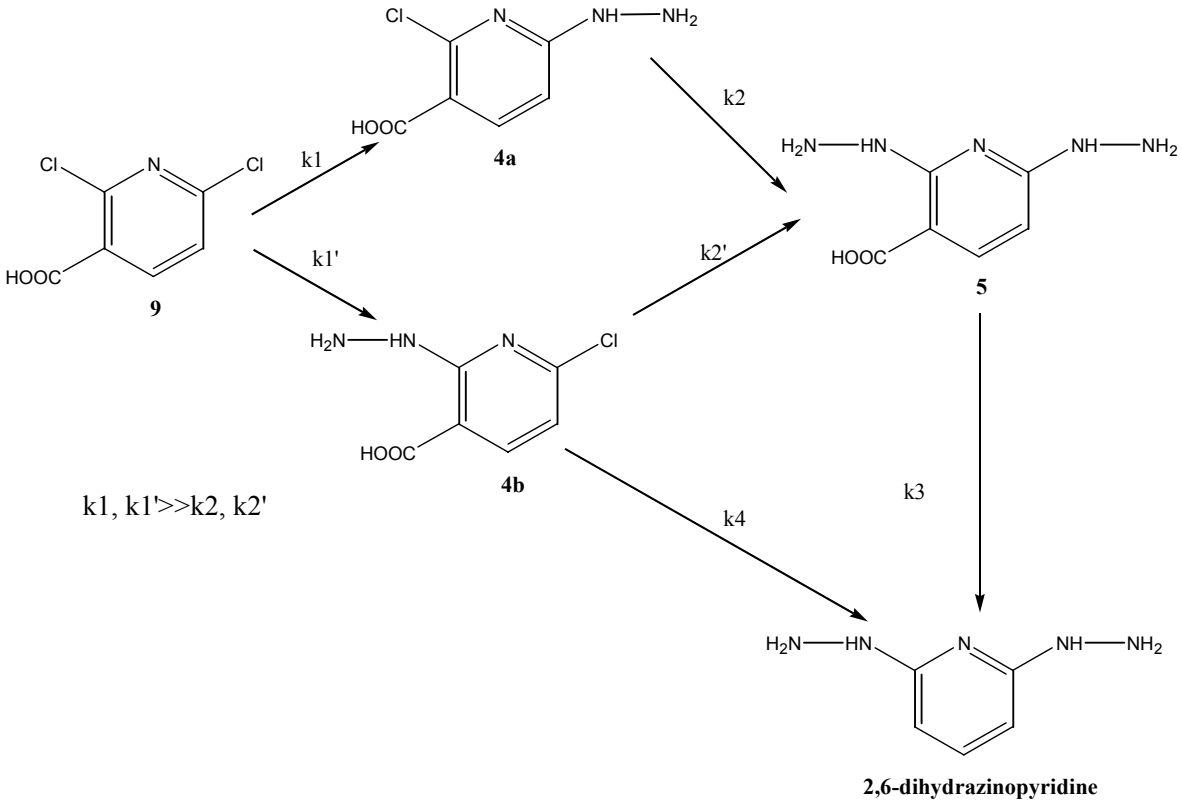
Time point	Mass Spectra (m/z in order of relative abundance)
t=0	188.0218 (100%), 184.0825 (50%), 190.0189 (33%), 140.0927 (5%)
t=20	140.0926 (100%), 184.0824 (73%), 188.0218 (35%), 190.0188 (10%)
t=40	140.0926 (100%), 184.0825 (25%), 188.0216 (10%), 190.0188 (3%)
t=60	140.0926 (100%), 184.0825 (15%), 188.0216 (5%)

Table E3: Mass spectra obtained at different time points during Experiment 2 (all compounds represented by $[M+H]^+$)

Discussion

Based on the obtained LC-MS data the monosubstitution of **9** to give **4a** and/or **4b** happens quickly; **9** could not be detected even at t=0. Thus we can conclude that the disubstitution of

9 to give **5** happens in two steps with **4a** and/or **4b** being intermediates. The putative mechanism for the dihydrazination of **9** (at T=100°C) is shown on Scheme E2:



Scheme E2: Putative mechanism for the dihydrazination and decarboxylation of 9

Since 2,6-dihydrazinopyridine was almost exclusively detected after 60 minutes the temperature (100°C) was higher than optimal.

Experiment 3

Results

Results of LC-MS analyses are summarised in Tables E4-5.

Peak	RT (min)	Area (%)
		t=1020
1	0.36	100
2	0.54	6.3
3	1.34	27.8

Table E4: HPLC data obtained for Experiment 3

Time point	LC peak	Mass Spectra (m/z in order of relative abundance)
t=1020	1-2	184.0827 (100%), 140.0926 (15%), 154.0605 (15%)*
	3	188.0216 (100%), 190.0186 (31%)
		*: probably fragment ion (N_2H_3 loss)

Table E5: MS data obtained during Experiment 3

Discussion

Results of LC-MS analyses suggested the presence of three compounds (peaks). The desired product (5) eluted with 2,6-dihydrazinopyridine in peaks 1-2 and a significant amount of **4a**/**4b** intermediate was still present (peak 3). Based on the obtained data, the applied conditions were too "mild", a conversion above 90% would be more appreciated to be able to avoid the need of purification. This experiment confirmed the putative mechanism described in Scheme E2 as **9** could not be detected in the sample (its conversion into **4a**/**4b** was 100%). The conversion of **4a**/**4b** to **5** was around 75%, with 2,6-dihydrazinopyridine already being present.

Experiment 4

Results

LC-MS data are summarised in Tables E6-7.

Peak	RT (min)	Area (%)	
		t=240	t=900
1	0.35	100	100
2	0.43-0.5	36.1	9.8
3	1.2-1.3	95.3	6.2

Table E6: LC data for Experiment 4

Time point	LC Peak	Mass Spectra (m/z in order of relative abundance)
t=240	1-2	184.0825 (100%), 140.0926 (9%)
	3	188.0217 (100%), 190.0188 (31%)
t=900	1-2	184.0826 (100%), 140.0928 (81%)
	3	Peak 3 was not present

Table E7: MS data for Experiment 4

Discussion

The above data suggest that the conversion is almost 100% at 60°C in 900 minutes as only **5** and 2,6-dihydrazinopyridine were detected at the end of the experiment. To summarise, the optimal conditions for making **5** are those applied in experiment 4: incubation of **9** in hydrazine hydrate at 60°C for at ~15 hours. Due to having/lacking (**5**/2,6-dihydrazinopyridine) the carboxylic acid moiety **5** and 2,6-dihydrazinopyridine could be separated easily.

4. ^{99}Tc and ^{99m}Tc labelling of 5 using the tricine-EDDA co-ligand system

Mass spectra of the above system showed two product ions at m/z= 613.0829 (a) and 615.0993 (b), corresponding to $C_{18}H_{27}N_8O_{10}{}^{99}Tc$ (a) and $C_{18}H_{25}N_8O_{10}{}^{99}Tc$ (b). The formula for ion (a) could either be **5** ($C_6H_9N_5O_2$)+2×EDDA ($C_6H_{12}N_2O_4$)+^{99}Tc-7H-NH or **5** ($C_6H_9N_5O_2$) +EDDA ($C_6H_{12}N_2O_4$)+tricine ($C_6H_{13}NO_5$)+^{99}Tc-7H-H_2O. The same was found for ion (b), *i.e.* the putative formula of (b) can either stand for **5** ($C_6H_9N_5O_2$)+2×EDDA ($C_6H_{12}N_2O_4$)+^{99}Tc-5H-NH or **5** ($C_6H_9N_5O_2$) +EDDA ($C_6H_{12}N_2O_4$)+tricine ($C_6H_{13}NO_5$)+^{99}Tc-5H-H_2O. Although a water loss seems to be more probable, the degradation of the hydrazine moiety could also occur (see reference 4 in main article).