Supporting Information

Study on the Accelerated *Gutknecht* Self-Cyclocondensation of Amino-sugars at Atmospheric Pressure Chemical Ionization Condition

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1. General Information

Commercial reagents, compounds and solvents were used without further purification. D-glucosamine hydrochloride (**1**·HCl), D-mannosamine hydrochloride (**2**·HCl), D-galactosamine hydrochloride (**3**·HCl), *N*-acetyl-D-glucosamine (**6**) and 4-methylumbelliferyl *N*-acetyl-β-D-glucosaminide (**7**) were purchased from Sigma-Aldrich (St. Louis, MO, USA) and used without further purification. Deoxyfructosazine[2-(arabotetrahydroxybutyl)-5-(erythro-2,3,4-trihydroxybutyl)pyrazine] (**4**) and Deoxyfructosazine [2-(arabotetrahydroxybutyl)-5-(erythro- 2,3,4-trihydroxybutyl)pyrazine] (**5**) were synthesized and purified according to the reference protocols (Rohovec, J.; Kotek, J.; Peters, J. A.; Maschmeyer, T. *Eur. J. Org. Chem.* **2001**, (20), 3899-3901). H_2O (HPLC grade, from Fisher Scientific, fair lawn, New Jersey, USA) and CH_3CN (HPLC grade, from Dikma Technologies, Lake Forest, USA) were purchased and applied for the preparation sample solution (in the mixing solvent of H_2O and CH_3CN (V:V=1:1)).

2. Mass spectrometric experimental conditions

The mass spectrometer instrument applied in this work is an ion-mobility quadrupole time-of-flight mass spectrometer (IM-Q-TOFMS, Agilent Technologies, Santa Clara, CA) with a drift tube coupled to a Q-TOF mass spectrometer. Both an Agilent atmospheric pressure chemical ionization (APCI G1947B) ion source and Agilent jet stream electrospray ionization Source (Dual AJS ESI G1958-65268) could be equipped for the IM-Q-TOFMS and also could be easily switched. Each mass spectra

were obtained by injection of the 1~5μl sample solution directly with the Agilent 1290 Infinity auto-sampler (G4226A) and the backgroup flow solution (ACN/H_2O:50/50) remained at 0.2 mL/min by 1290 binary pump (G4220A). Dual AJS ESI source conditions were: spray voltages of 3500V for capillary entrance and 500V for nozzle, Nitrogen sheath gas temperature (100~250°C) at a flow rate of 12 L/min, Nitrogen drying gas was 150°C at a flow rate of 10 L/min, Nitrogen nebulizer at 35 psig. APCI source conditions were: spray voltages of 3500V for capillary entrance and Corona current 4μA, vaporizer temperature of 350°C, Nitrogen drying gas was 150°C at a flow rate of 7 L/min, Nitrogen nebulizer at 35 psig. Both APCI and ESI source were operated in positive mode. The ESI spray probe could be easily replaced by APCI operation.

Ion mobility (IM) separation was achieved in a uniform field drift tube. The high purity nitrogen (99.999%) was used as the buffer gas at pressure of 3.98 Torr and 25 °C in the drift tube and the drift entrance voltage was set as 1700 V. The ion mobility separation capacity remained at resolving power of 60 ($t/\Delta t$, t=drift time) approximately. IM was operated at frame rate 0.9 frames/s, IM transient rate 13 IM transients/Frame, max drift time 80 ms and TOF transient rate 667 transient/IM Transients. The Q-TOFMS (Agilent 6560A) with mass resolution about 40000 (FWHM) could perform the high resolution measurements and collisional induced dissociation experiments at same time. IM-MS spectra with mass range between 50 *m/z* and 1000 *m/z* were acquired with Agilent MassHunter workstation Data Acquirement software (LC/MS Data Acquisition for 6560 IM-QTOF, Version

B.06.00 Build 6.00.6032, Agilent Technologies). The mass measurement was calibrated externally using the Agilent tuning mixture solution. IM-MS spectra were viewed and analyzed by IM-MS Browser (Version B.06.00, Build 6.0.27.0, Agilent Technologies).

3. Mass Spectra and MS/MS mass spectra

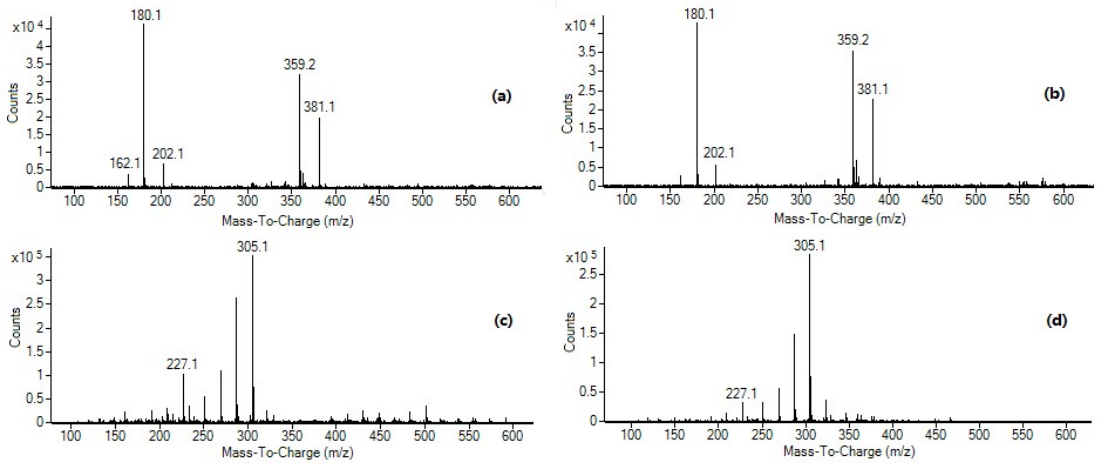
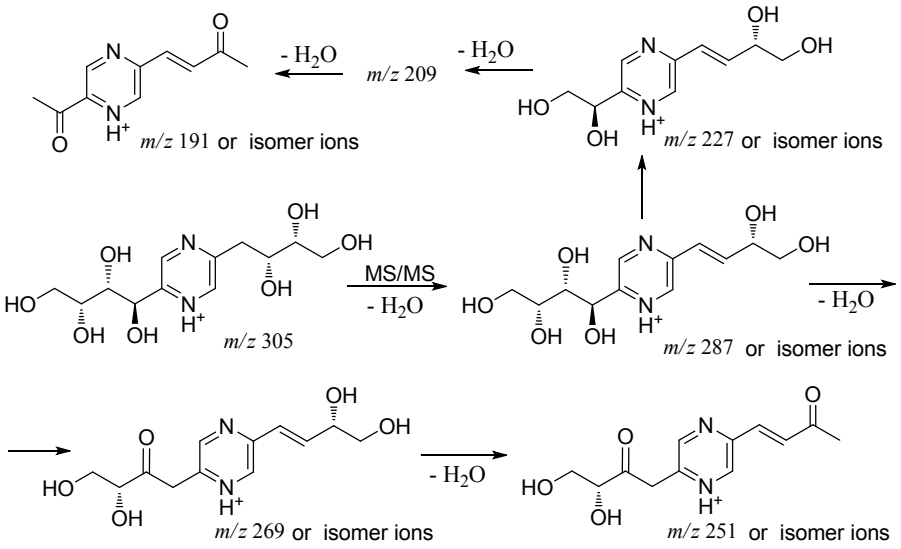


Fig. S1 (a) ESI-IM-Q-TOF-MS spectrum of D-mannosamine hydrochloride (**2**·HCl); (b) ESI-IM-Q-TOF-MS spectrum of D-galactosamine hydrochloride (**3**·HCl); (c) APCI-IM-Q-TOF-MS spectrum of D-mannosamine hydrochloride (**2**·HCl); (d) APCI-IM-Q-TOF-MS spectrum of D-galactosamine hydrochloride (**3**·HCl).

Table S1. Accurate mass determinations for the major ionic species of the amoni-sugars by APCI-IM-Q-TOF MS

Compounds	Calculated elemental composition of ions	detected *m/z*	theoretical *m/z*
D-glucosamine	$C_{12}H_{21}N_2O_7^+$	305.1350	305.1343
hydrochloride	$C_{12}H_{19}N_2O_6^+$	287.1237	287.1238

D-mannosamine	$C_{12}H_{21}N_2O_7^+$	305.1347	305.1343
hydrochloride	$C_{12}H_{19}N_2O_6^+$	287.1242	287.1238
D-galactosamine	$C_{12}H_{21}N_2O_7^+$	305.1339	305.1343
hydrochloride	$C_{12}H_{19}N_2O_6^+$	287.1234	287.1238



Scheme S1. The proposed fragmentation patterns of ion **5**·H^+ at *m/z* 305 in MS/MS.

The compounds **4** and **5** have many hydroxyl groups, therefore the fragment ions of **4**·H^+ or **5**·H^+ at *m/z* 305 caused by subsequent dehydrations might exist isomer ions.

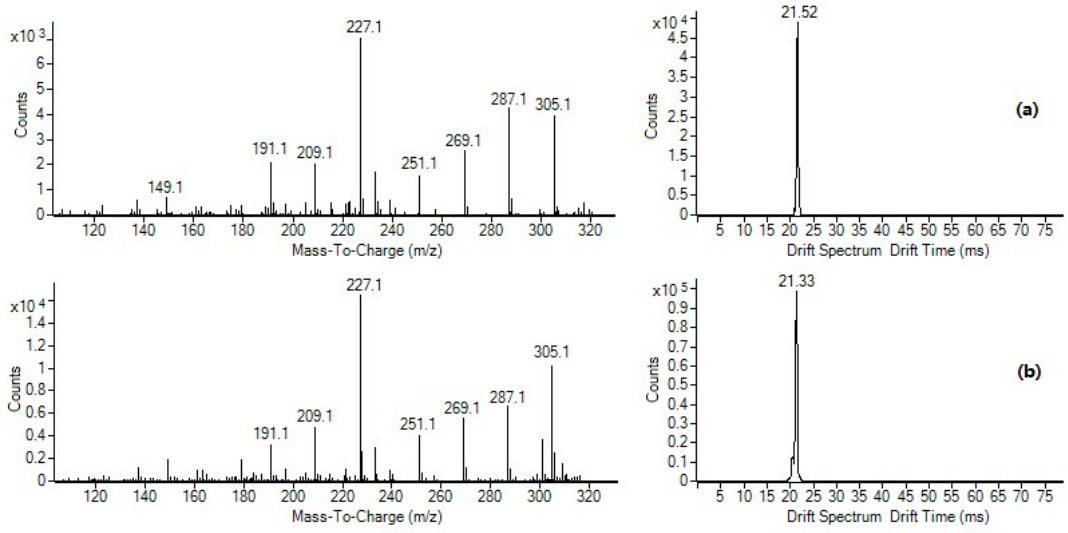


Fig. S2 (a) MS/MS spectrum of ion **4**·H^+ at *m/z* 305 from ESI-IM-Q-TOF-MS

analysis of **4** and its drift time spectrum; (b) MS/MS spectrum of ion **5**·H^+ at *m/z* 305 from ESI-IM-Q-TOF-MS analysis of **5** and its drift time spectrum.

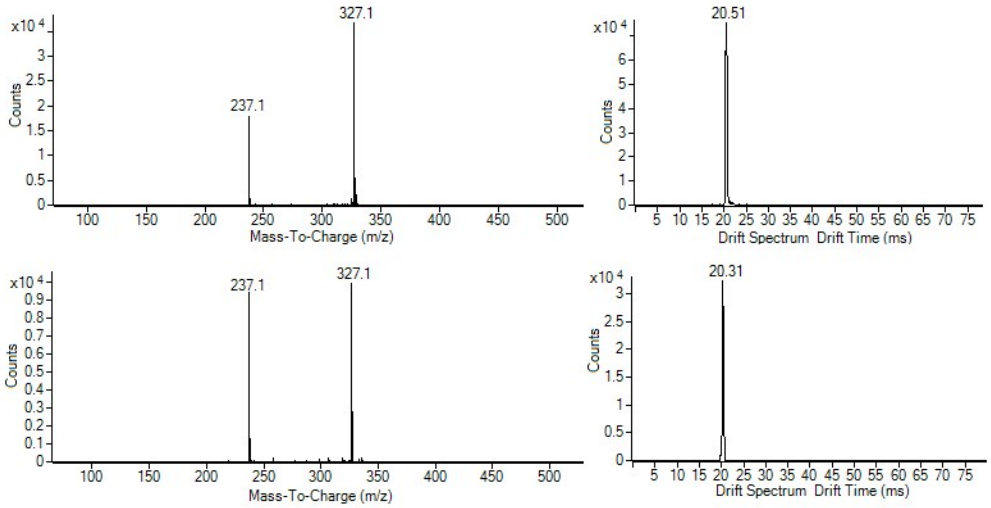
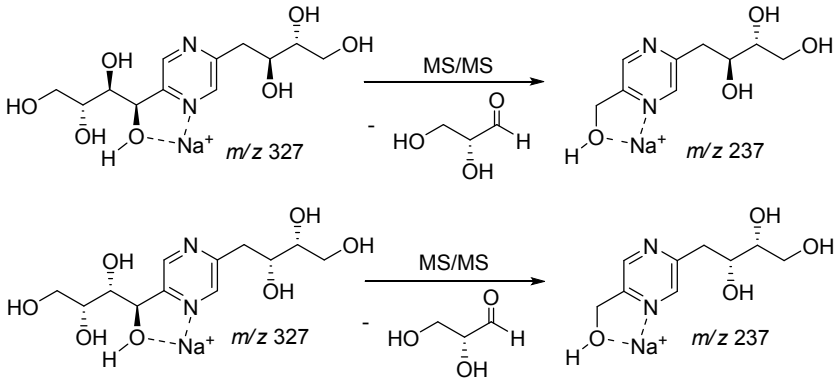


Fig. S3 (a) MS/MS spectrum of ion **4**·Na^+ at *m/z* 327 from ESI-IM-Q-TOF-MS analysis of authentic **4** and its drift time spectrum; (b) MS/MS spectrum of ion **5**·Na^+ at *m/z* 327 from ESI-IM-Q-TOF-MS analysis of authentic **5** and its drift time spectrum. The shorter drift time of **4**·Na^+ (20.51 ms) than **4**·H^+ (21.52 ms, Fig. S2) suggested that the configuration of **4**·Na^+ might be more compact than **4**·H^+. The results demonstrated that the slight differences in both MS/MS fragmentation patterns and the drift time experiment results between protonated/soudium caionized **4** and **5** could be distinguished at our experimental conditions.



Scheme S2. The proposed fragmentation patterns of ion **4**·Na^+ and **5**·Na^+ at *m/z* 327.

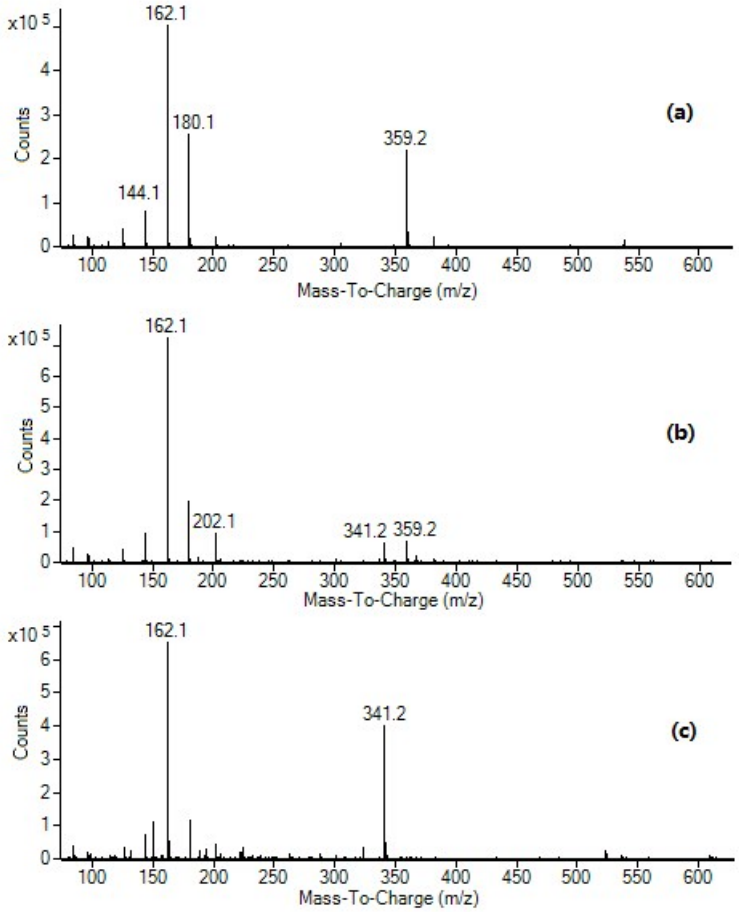


Fig. S4 ESI-IM-Q-TOF-MS spectra of D-glucosamine hydrochloride (**1**·HCl) with different sheath gas temperature of: (a) 250°C, (b) 300°C, (c) 350°C.

4. Sample collection in APCI-MS experiments

In order to test the possibility of applying APCI-based thermospray as a new synthesis method for the synthesis 2,5-DOF from D-glucosamine hydrochloride without applying any other catalyst, we designed a surface collection method to accumulate enough amount of product from the direct APCI-thermospray synthesis for further MS and ^{1}H NMR analyses by infusing high concentration D-glucosamine hydrochloride water solution to the APCI-ion source. In such surface collection

method, we used a small piece of aluminium foil to cover the orifice of the ion transfer line mass spectrometry to accept the products from the APCI-based thermospray synthesis and also prevent too much ions going the mass spectrometer (Fig. S5a). At same time we infused 1ml high concentration of hydrochloride D-glucosamine (about 16mg/ml in water solution) by syringe pump (PHD Ultra, Harvard Apparatus) at flow rate of 0.1ml/min. The APCI source conditions were similar with normal APCI measurements with the vaporizer temperature at 325°C. After APCI-thermospray experiments, some brown samples could be observed in the surface of aluminium foil (Fig. S5b), the tip of corona discharge needle and the surface of APCI source.

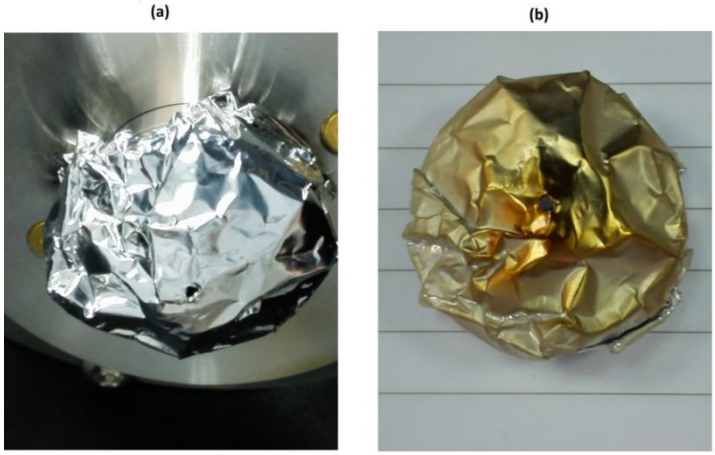


Fig. S5 (a) Aluminium foil used for surface collection of APCI-generating products, covering the orifice of the ion transfer line mass spectrometry; (b) after the sample collection steps, aluminium foil was covered by the products formed in the APCI-thermospray process.

We carefully washed the small amount of solid residues by using H_2O and analyzed

these sample solution by ESI-MS. Strong signals of 2,5-DOF could be observed from the solution of the collected samples in APCI-ion source (Fig. S6). The APCI-thermospray synthesis could performed twice to collect enough samples for ^{1}H NMR experiments and D_2O could be used directly as solvent to wash and collect products from aluminium foil in APCI-thermospray experiments (Fig. S5b). ^{1}H NMR analysis (Fig. 5 and Fig. S7) showed that the surface-collected unpurified samples contained 2,5-DOF. These results confirmed that the APCI-based *Gutknecht* self-condensation is not only an interesting ambient gas phase reaction but also a promising strategy for developing practical synthesis method of 2,5-DOF. The next step of our researches is to design and set up a more practical and efficient device to achieve the *Gutknecht* synthesis of 2,5-DOF from D-glucosamine hydrochloride by mimicking the APCI-thermospray conditions.

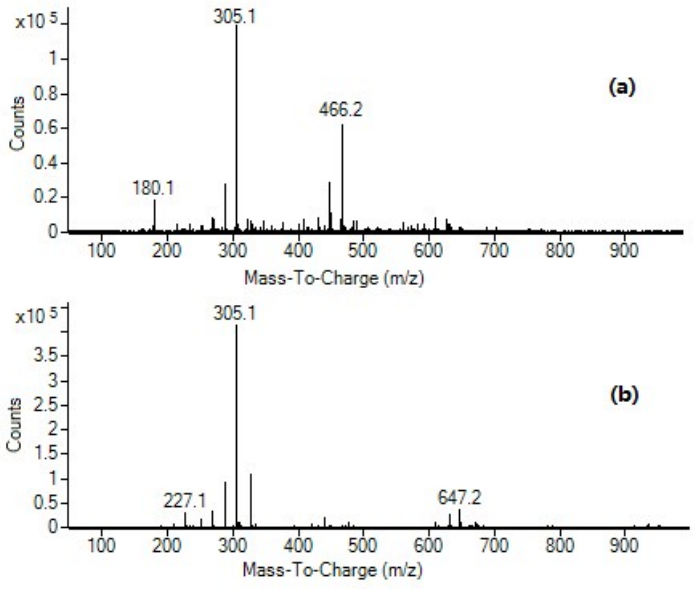


Fig. S6 (a) ESI-MS spectrum of unpurified samples collected from the APCI-thermospray synthesis, showing strong signal of *m/z* 305 and some unreacted D-

glucosamine hydrochloride giving signal at *m/z* 180; (b) ESI-Q-TOF-MS spectrum of authentic 2,5-DOF (**4**), showing the signal **4**·H^+ at *m/z* 305.

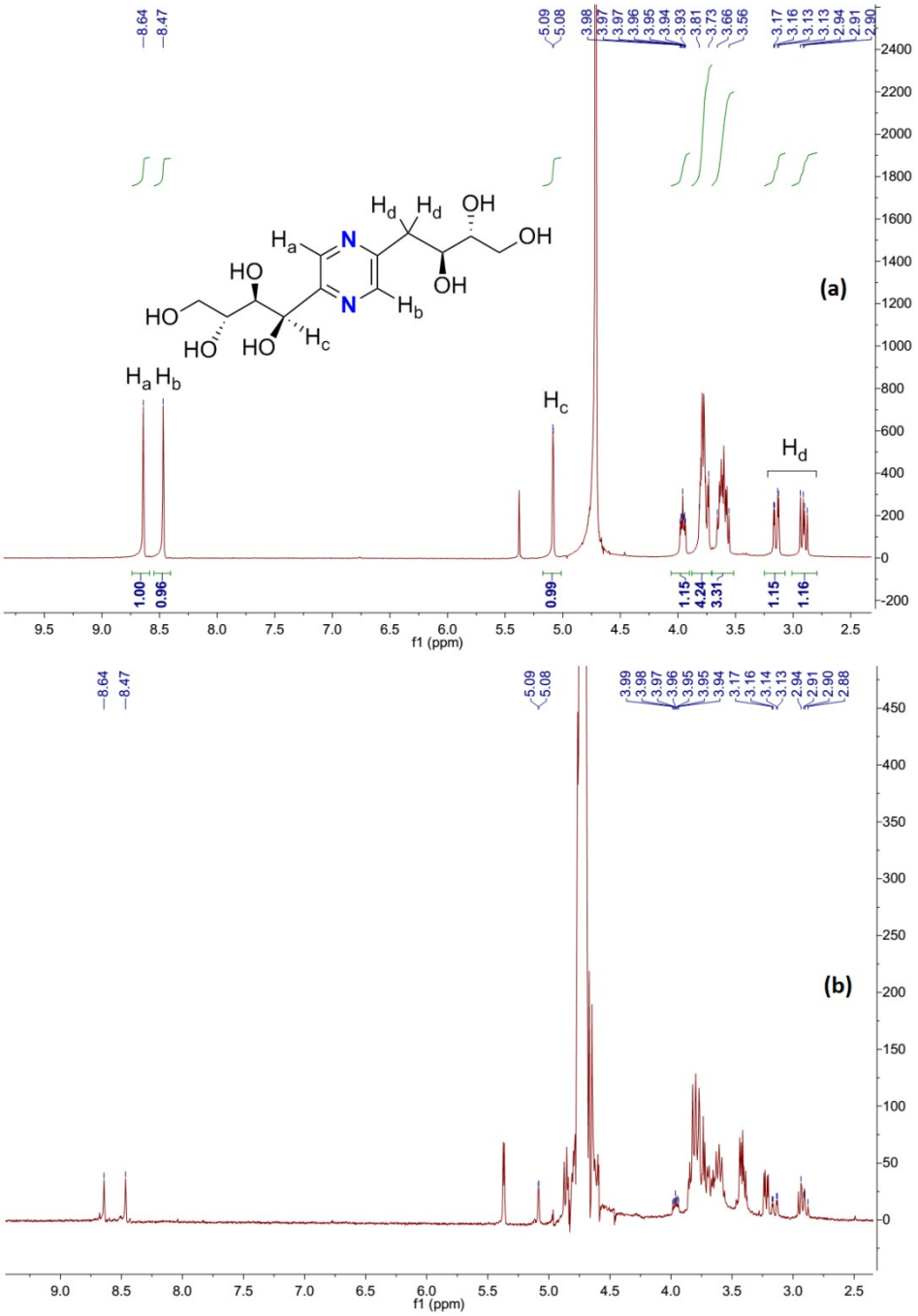


Fig. S7 The expanded ^{1}H NMR spectra (400MHz, D_2O) of: (a) the authentic 2,5-DOF (**4**) by traditional synthesis methods using phenylboronate as catalyst; (b) unpurified samples collected from APCI-thermospray synthesis.

Notice: At normal mass spectrometric measurement conditions, it is not recommended to infuse 1 mL such high concentration water solution of D-glucosamine hydrochloride (about 16mg/ml). That might heavily contaminate the APCI or ESI ion-source. In this article, the APCI-synthesis sample collection experiment was performed very carefully by covering the orifice of mass spectrometry with aluminium foil. After the APCI-synthesis sample collection experiments, every parts of APCI ion source should be completely cleaned up.