Electronic Supplementary Material (ESI) for Reaction Chemistry & Engineering.
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Supporting information to Accompany:

Mechanistic Insight *into* Oxidized *N,N*-Dimethylacetamide as a source of Formaldehyde Related Process Derivatives

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1. **Chemicals and Reagents**: Formaldehyde (37% aq), CD_3CN and D_2O, CH_3CN, MTBE, $MgSO_4$, t-BuOOH, NMA were purchased from

Sigma-Aldrich. DMAc was purchased from three sources (Sigma Aldrich, Fisher, Fluka). PBN was purchased from Sigma-Aldrich.

2. UPLC/MS method: Acquity UPLC BEH C18 1.7 um 100x2.1mm column, Temp = 40 oC, flow rate = 0.6 mL/min, UV = 210, Time = 15 min, post time = 3 min. Mobile Phase A = 2 mM Ammonium Formate (pH ~8.6), Mobile Phase B= ACN. Gradient Time = 0min, %B = 5, Time = 3min, %B = 4, Time = 13min, %B = 95, Time = 15 min, %B = 95, Time = 15.1 min, %B = 5. Sample concentration = 0.5 mg/mL, inj volume = 2uL. The MS analysis was performed using an Water ACQUITY QDa Mass Detector.

3. Instrumentation for MS/MS analysis: HPLC/UV: LC/MS analysis was performed on a Waters Acquity UPLC system, consisting of a binary pump system, a sample manager, a PDA detector, and a Waters Premier Mass Spectroscopy (Waters, Milford, MA) under positive or negative ESI conditions. The output signal was monitored and processed using MassLynx software designed by Waters (Milford, MA). The separation was carried out on a Waters BEH C18 column (2.1 x 50 mm, 1.7 µm particle sizes). The mobile phase consisted of water with 0.1% formic acid (mobile phase A) and acetonitrile (mobile phase B). The injection volume was 3 µL. Analytes were eluted using a gradient method consisting of an initial hold at 1% mobile phase B for 0.5 min, followed by a linear gradient to 90% B over 3.5 min, then a linear gradient to 0% B over 0.1 min, a hold at 0% B for 0.4 min, next a linear gradient to 10% B for 0.01 min, and finally a hold at 10% B for 0.49 min for a total run time of 5 minutes. The flow rate was 0.5 mL/min. The column temperature was maintained at 40°C. The PDA detector was set at 200-400 nm.

Mass Spectrometry: The eluent was introduced directly into the mass spectrometer via the electrospray ionization source. Mass spectrometry was performed on a Waters Premier Q-ToF operating in positive ion mode. Source temperature and desolvation temperature were set at 120°C and 400°C, respectively. Nitrogen was used as both the cone gas (50 liters/h) and desolvation gas (800 liters/h). The capillary voltage was set to 3 KV. The cone voltage applied was 10 V. Leucine enkephalin was used as the lock mass (m/z of 556.2771) for accurate mass calibration and was introduced using the lock spray interface at 20 µl/min at a concentration of 0.5 mg/mL in 50% aqueous acetonitrile containing 0.1% formic acid. During mass spectrometry scanning, data were acquired in the centroid mode from *m/z 5*0 to 1000.

4. Analysis of N-Methyl Acetamide (NMA) in DMAc by GC:

Chromatographic Conditions: Agilent CP-Sil 5 CB, 25 m x 0.25 mm, 1.2 um fil thickness. Temp. Program=105 °C for 1.5 min, 10 °C/min to 150 °C, 30 oC/min to 240 °C and hold for 3 min. Run Time = 12 min. Oven eqilibration Time = 1 min. Injector= 240 °C, split ratio 25:1. Detector=FID at 240 °C. Carrier Gas = helium, constant Flow rate = 1.3 mL/min.

Preparation of Solutions: Diluent: 1,3-Dimethyl-2-Imidazolidinone (DMI)

Standard: Prepare a 1.0% v/v stock standard solution by pipetting 1.0 mL of NMA into a 100 mL volumetric flask. Dilute to volume with diluent. Prepare 0.01% v/v standard solution by pipetting 1.0 mL of the 1.0% v/v stock standard solution into a 100 mL volumetric flask. Dilute to volume with diluent. The 0.01% v/v standard solution will be used for quantitation.

System Suitability Solution (SST): Pipette 0.5 mL of the 1.0% v/v stock standard solution into a 100 mL volumetric flask. Dilute to volume with diluent. This is SST solution (0.005% NMA solution).

Sample: Pipette 1.0 mL of DMAc into a 10 mL volumetric flask. Dilute to volume with diluent.

Retention Time and Density Table

Solvent	Retention Time (min)	Density (g/mL)
N-methylacetamide (NMA)	3.28	0.957
N,N-dimethylacetamide (DMAc)	3.96	0.937

System Suitability

1. The signal to noise ratio for the NMA peaks in the System Suitability Solution should be ≥10.
2. The chromatography should be similar to that illustrated in Figure 1.
3. RSD of area counts for at least 5 injections of the 0.01% v/v standard solution should be ≤ 10%.

Calculations:

$$\text{Volume \% Solvent} = \frac{(Ax)(Cs)}{(As)}(DF)$$

A_x = Peak area in the sample

A_s = Average peak area in the standard

C_s = Concentration of the standard in volume %

DF = Dilution Factor of DMAc Sample in diluent

$$\text{Weight \% Solvent} = \frac{(Ax)(Cs)(\rho)}{(As)(d)}(DF)$$

5. GC/MS chromatogram of a "bad" DMAc sample

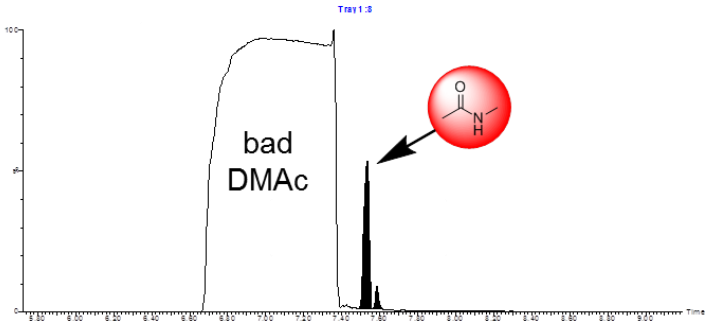


Figure S1. "Bad" DMAc directly injected on GC shows prersence of N-methylacetamide

6. MS/MS data for *hemiaminal* impurity in Ceftolozane

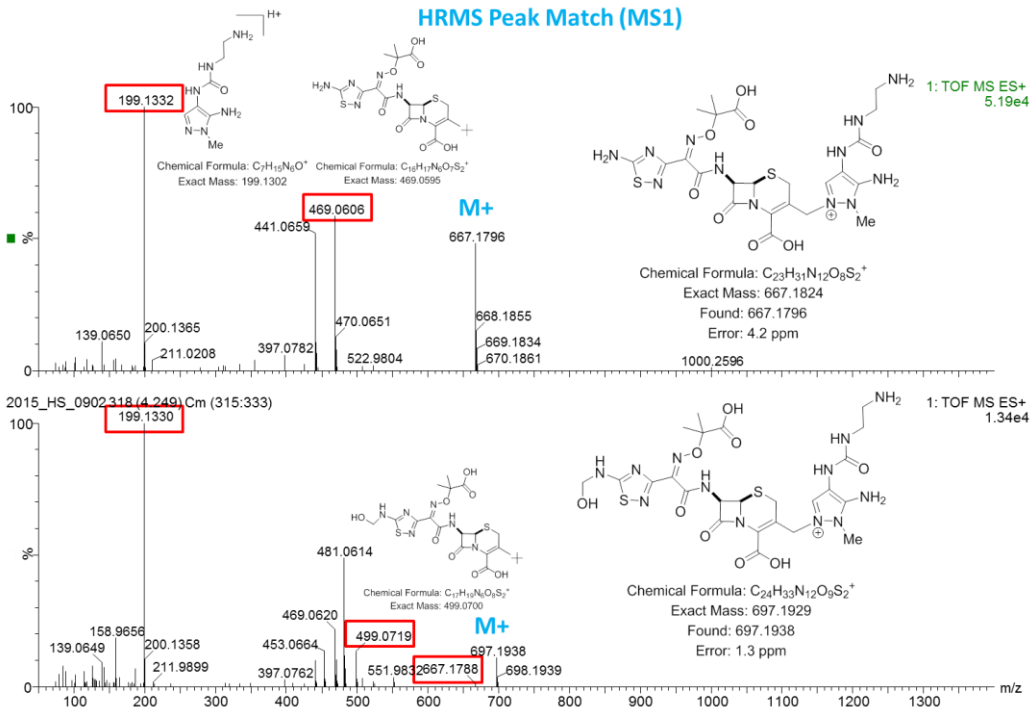


Figure S2. The HRMS Spectra of parent (**1**) with m/z 667 and formaldehyde adduct (**2**) with m/z 697. The m/z 469 and 499 clearly indicated that the CH2O addition occurred on the left part of the molecule.

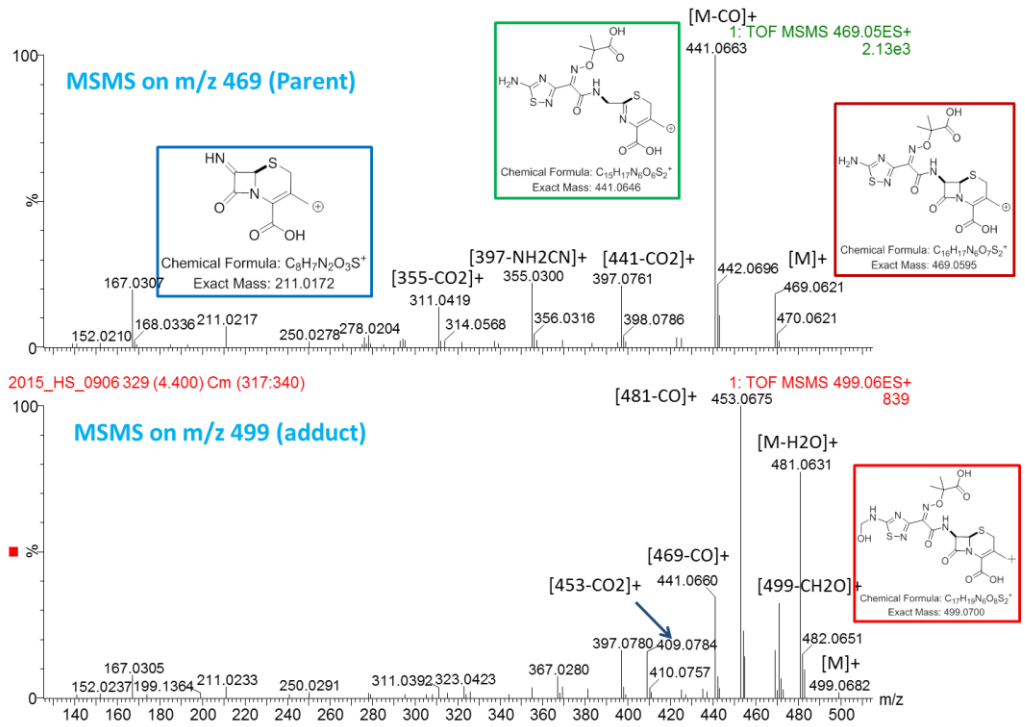


Figure S3. The HRMS/MS Spectra of parent fragment ion with m/z 469 and formaldehyde adduct fragment ion with m/z 499. The result indicated the CH2O was added on the left part of the m/z 469 ion.

7. MS/MS data for carbon bridged product generated from 13C labeled and oxidized DMAc

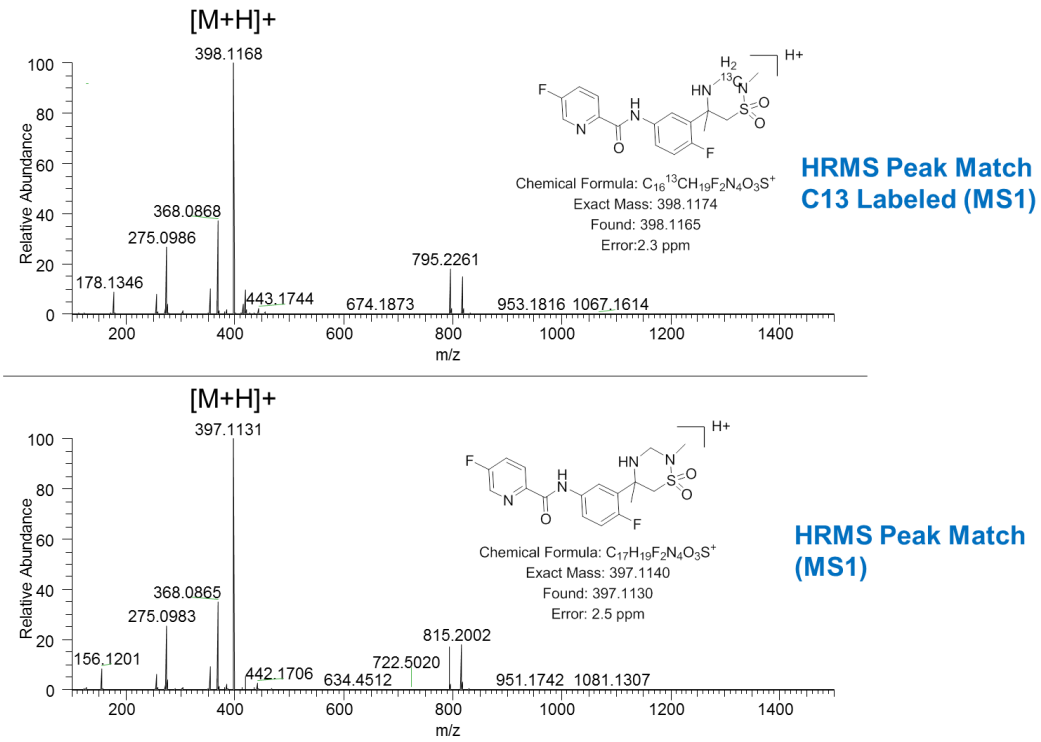


Figure S4. The HRMS Spectra of parent (**11**) with m/z 397 and C13 labeled parent (**12**) with m/z 398.

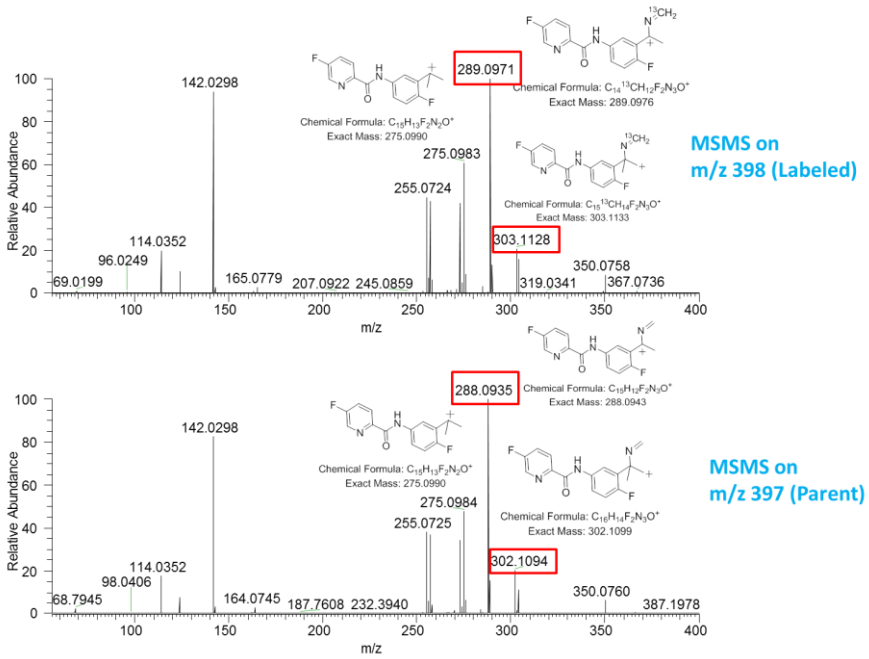


Figure S5. The HRMS/MS Spectra of parent (?) with m/z 397 and C13 labeled parent with m/z 398. Fragment ion m/z 302, 303 and m/z 288, 289 clearly indicated the position of the C13 labeled carbon.

8. Overlay GC chromatogram showing level of N-methylacetamide in selected DMAc samples

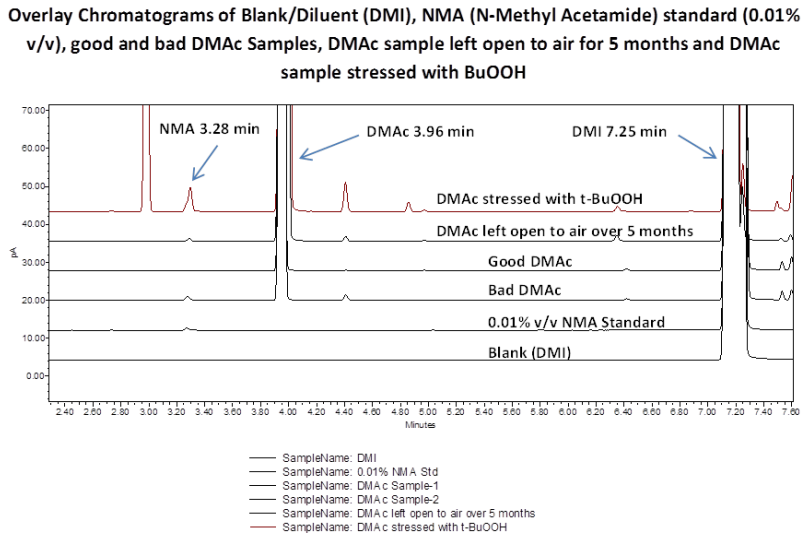


Figure S6. GC chromatogram showing various levels of N-methylacetamide

9. Impurity control strategy based on correlation between N-methylacetamide in DMAc and aminal impurity in Ceftolozane

Area % of aminal impurity Peak in API	NMA Limit in DMAc (wt%)
0.05	0.047
0.045	0.042
0.04	0.037

Table S1: correlation between wt% of NMA in DMAc vs HPLC area% of monoaminal impurity in API

Description of control strategy: Based on batch data and multiple lab runs, there is an average rejection of 40-60% for the mono aminal impurity during DMAc solvate crystallization. This suggests that DMAc solvate with < 0.10 area% aminal impurity can be rejected to below the 0.05 area% during manufacturing process. Experimental results show 0.06 area% mono aminal adduct in DMAc solvate was reduced to 0.026 area% during crystallization. Controlling the level of NMA below 0.13area % does control the level of mono aminal impurity to less than 0.10 area%.

10. LC/MS data for oxidized DMAc showing generation of hydroxy DMAc derivatives

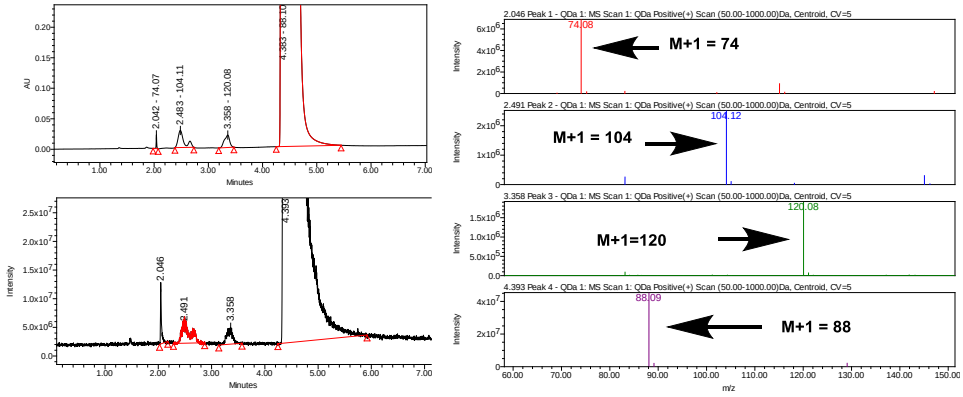
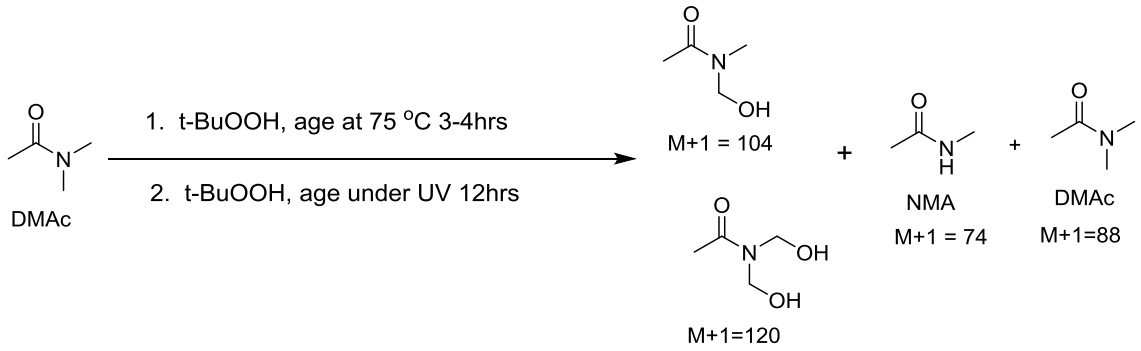


Figure S7. GC/MS data showing generation of NMA and highly polar impurities generated in oxidized DMAc

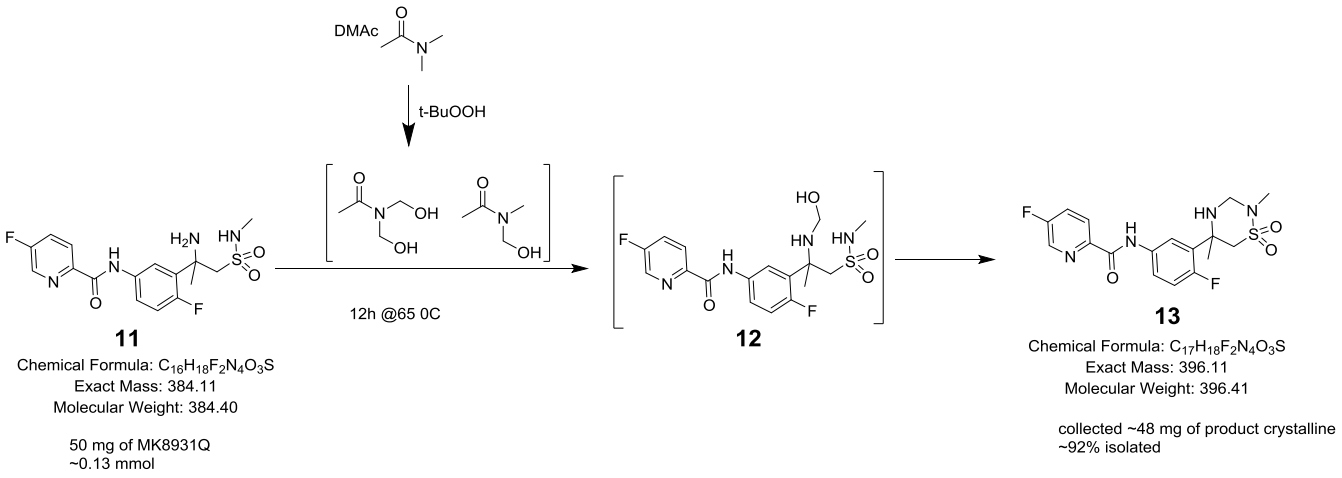
11. Protocol used to generate oxidized DMAc



Scheme S1: oxidation of DMAc with t-BuOOH at hight temperature or under UV generating NMA and oxidized species
Generation of Bad DMAc from Good DMAc: 1. Clean DMAc (15 mL, 14.1g, 83.3 161.8 mmoles) with less than 0.05A% of NMA by GC is treated with 2 mL t-BuOOH (70wt% aq, 1.4 g t-BuOOH, 15.5 mmol) and mixture is aged at 75 oC overnight. 2. Alternatively oxidation of DMAc can be accomplished if mixture is aged under UV light overnight. The oxidized DMAc can be stored at RT and used for generation of hemiaminal derivatives.

12. Protocol used to generate carbon bridged product:

(a) Oxidation of DMAc. DMAc (1.5 mL, 1.41g, 16.2 mmol) was treated with t-BuOOH (0.2 mL, 70% aq, 0.13g t-BuOOH, 1.5 mmol) and reaction mixture aged at 75 °C over 4 hrs. This DMAc was directly used for subsequent carbon bridge derivatization reaction

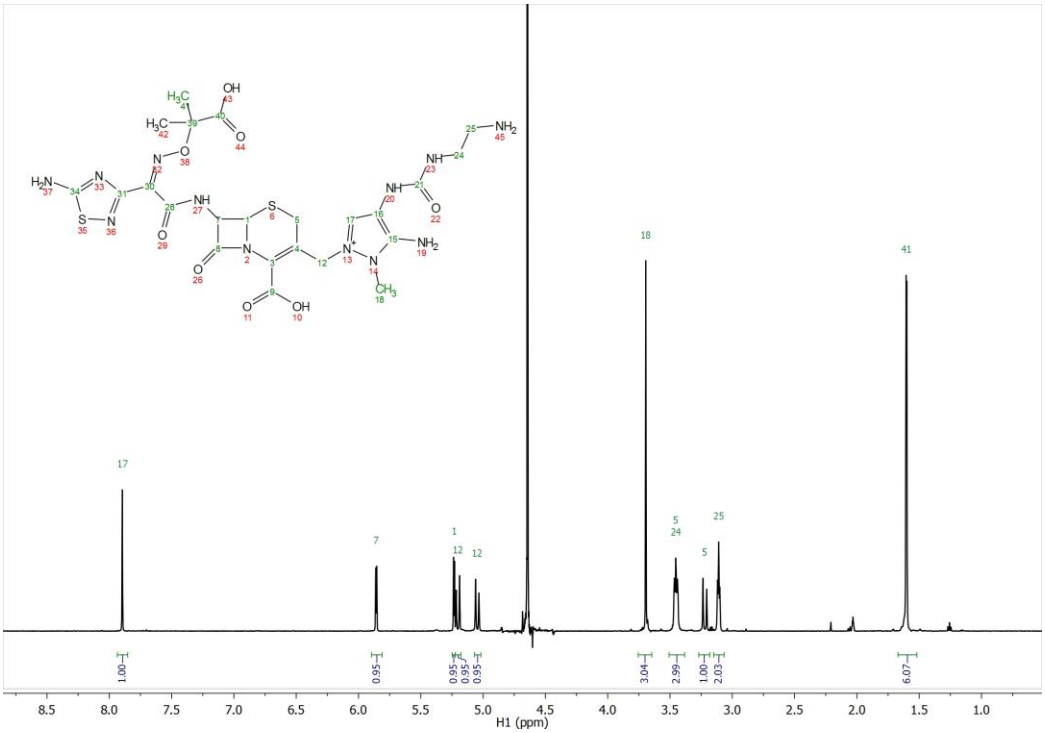


Scheme S2: Oxidized DMAc generating carbon bridged product

(b) Starting material 11 (50mg, 0.13mmol) was treated with oxidized DMAc (1.5 mL) and reaction mixture was aged overnight at 75 °C. HPLC analysis shows 98% conversion after overnight. Add 20 mL of water followed by 10 mL of MTBE and stir mixture for over 10 min. MTBE layer was collected and subjected to $MgSO_4$ drying, filtered and washed with 5 mL of MTBE. Organic layer was then concentrated over rota-vap affording 48 mg of solid 13 (0.12 mmol, 92.3% isolated yield) LC/MS and NMR studies confirm carbon bridge formation in 13. Same protocols described in (a) and (b) were used to generate ^{13}C bridged derivative 10.

13. NMR conditions: NMR data were collected at 25°C on a 500MHz Bruker instrument equipped with a liquid N2 cooled Prodigy™ probe. The proton spectra of compound 10 of natural abundance (Fig 3a) and compound 10 generated with ^{13}C DMAc (Fig 3b) were both acquired for one transient with a recycling delay of 5 s and an acquisition time of 2.70s. The ^{13}C decoupled proton spectra of compound 10 (Fig 3c) was acquired for one transient with a recycling delay of 5 s, an acquisition time of 0.18 s, and a 3571 Hz GARP decoupling sequence.

14. Assigned NMR spectra of parent (1).



a)

b)

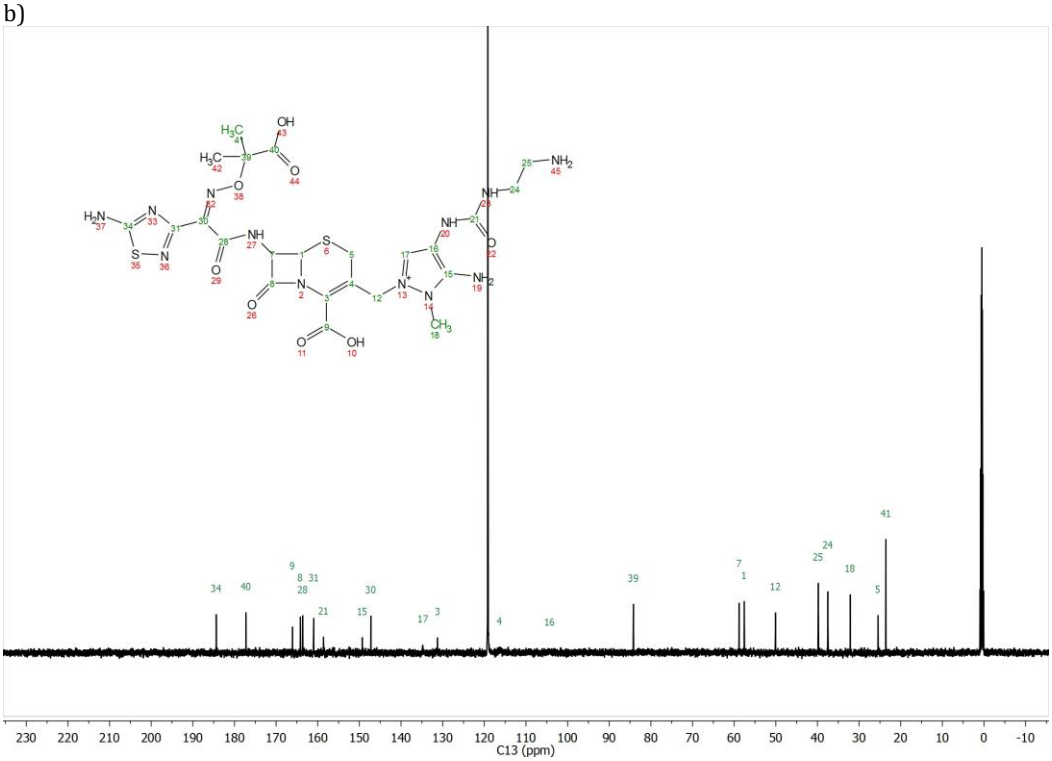
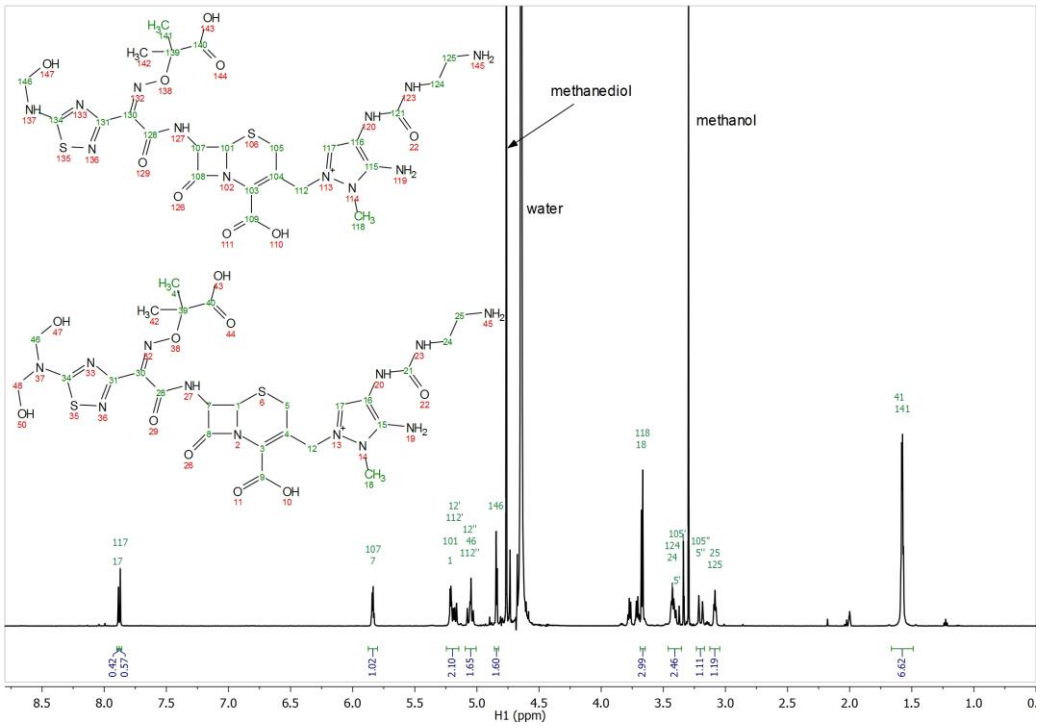


Figure S8:

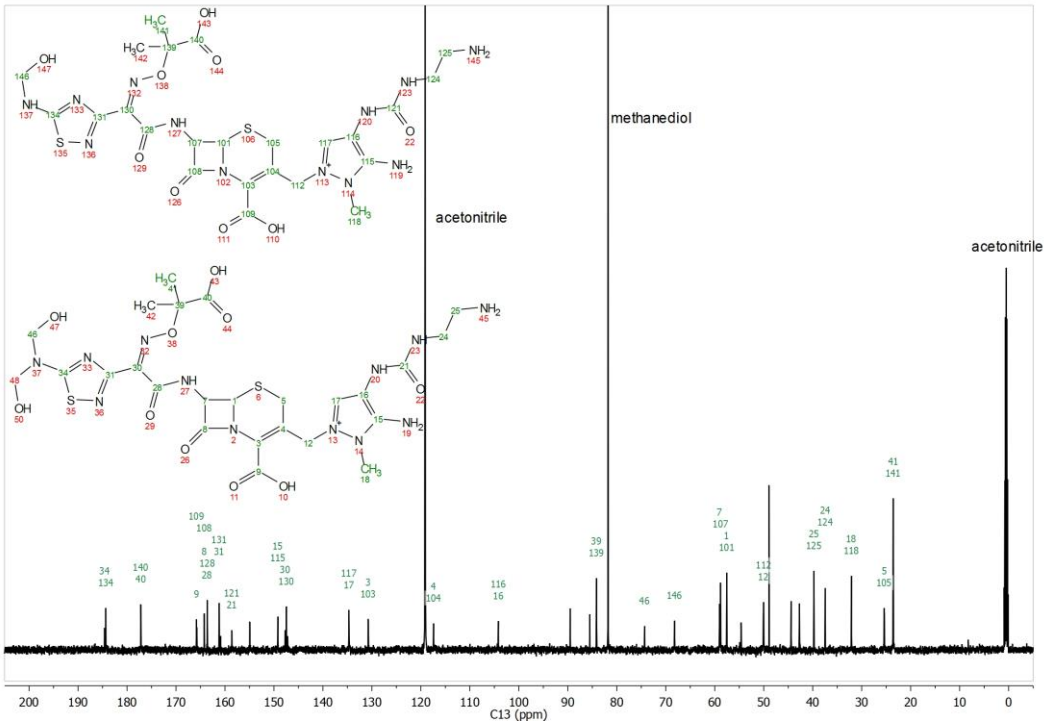
a) ^{1}H NMR spectrum of parent (1) in D_2O:CD_3CN 5:1 mixture; b) ^{13}C NMR spectrum of parent (1) in D_2O:CD_3CN 5:1 mixture.

15. Assigned NMR spectra of aminal (maj2, or, ~60%) and bis aminal (minor, ~40%) impurities.

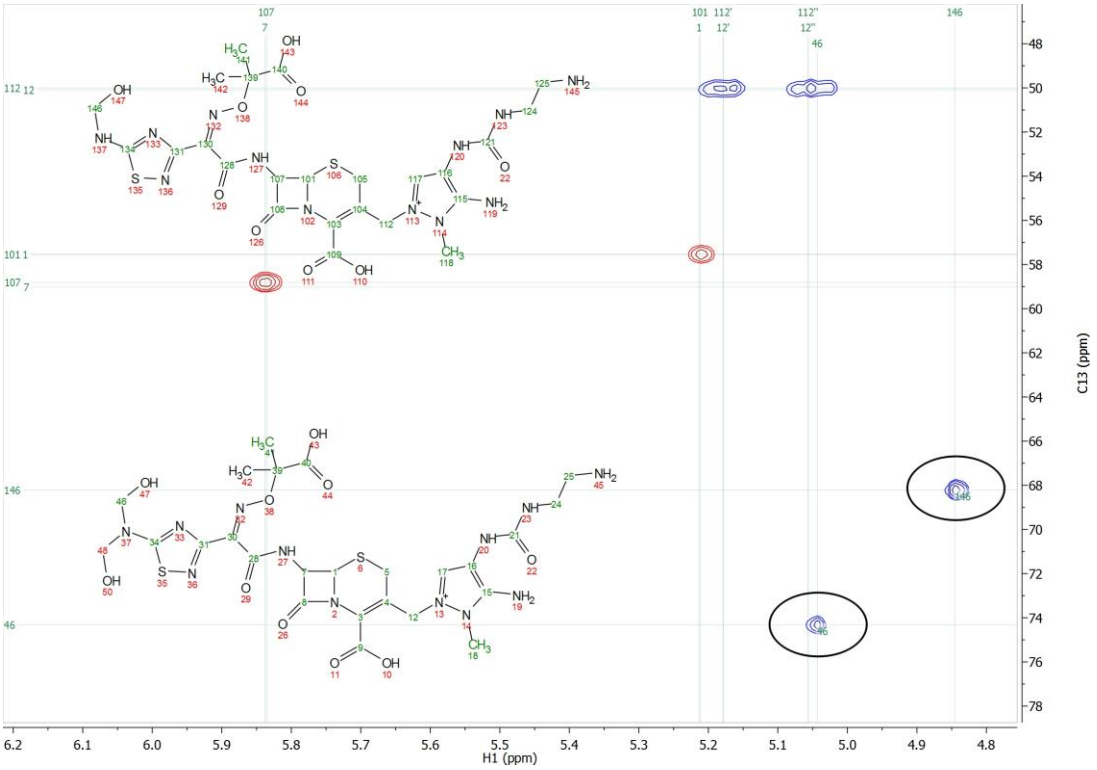
a)



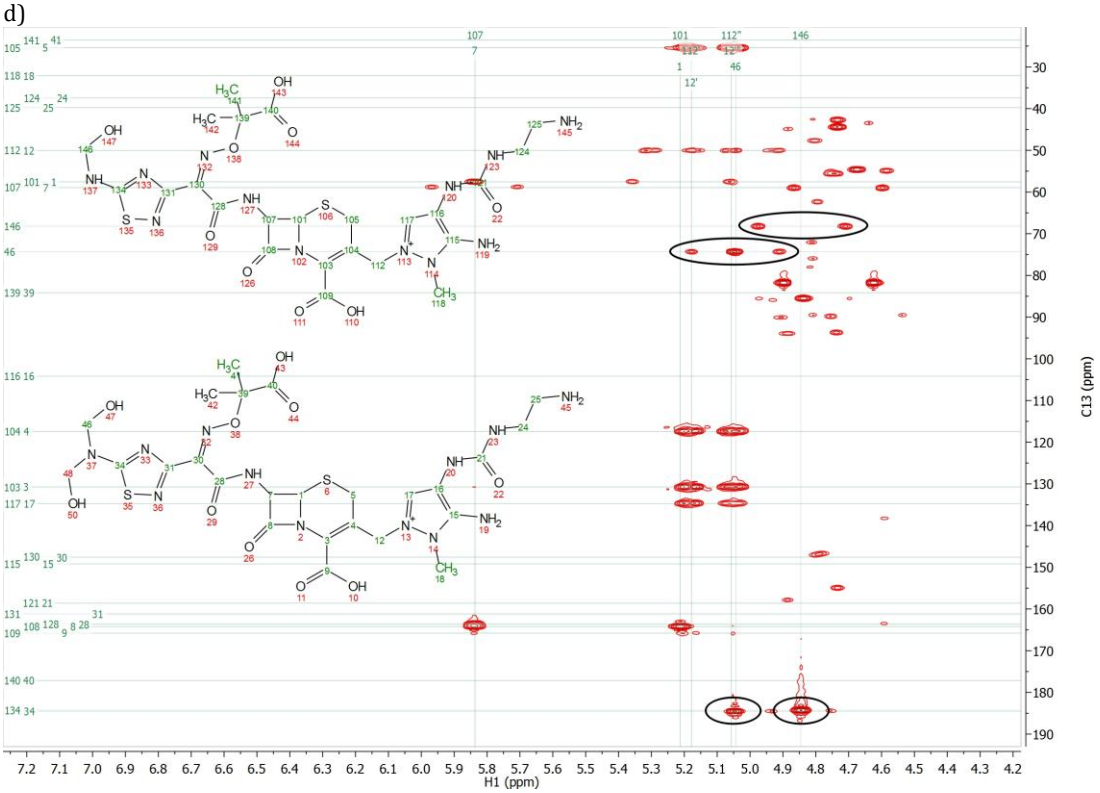
b)



c)

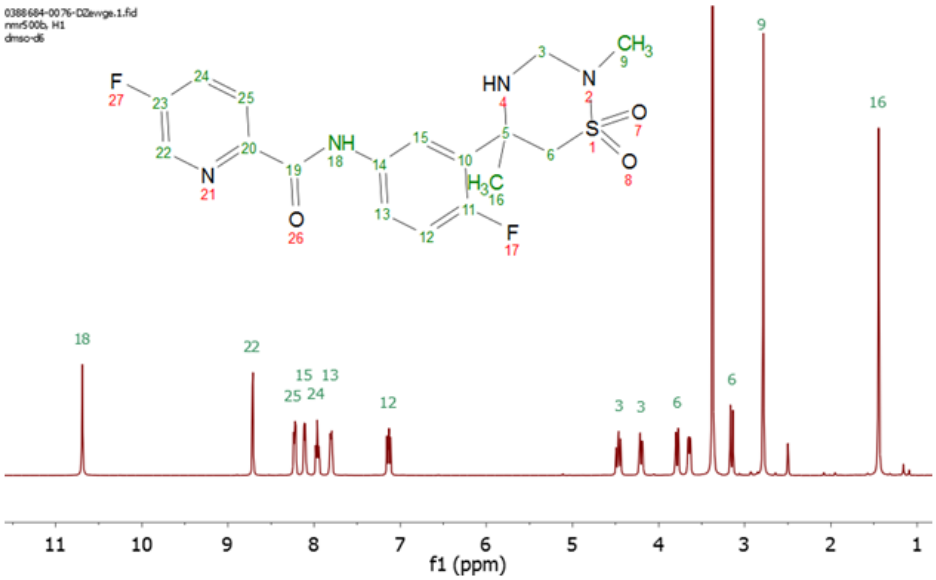

C13 (ppm)
H1 (ppm)

d)



All NMR spectra are acquired in in D_2O:CD_3CN 5:1 mixture.
Figure S9: a) ^1H NMR spectrum. b) ^{13}C NMR spectrum. c) ^1H-^{13}C multiplicity-edited HSQC spectrum shows that the biggest difference between the two molecules is in the ^1H and ^{13}C chemical shifts of the aminal adducts. d) ^1H-^{13}C HMBC spectrum shows key evidence that both mono- and bis- adducts are formed on the thiadiazole amine moiety.

16. ^{1}H and ^{13}C NMR for ^{13}C bridged product 13:



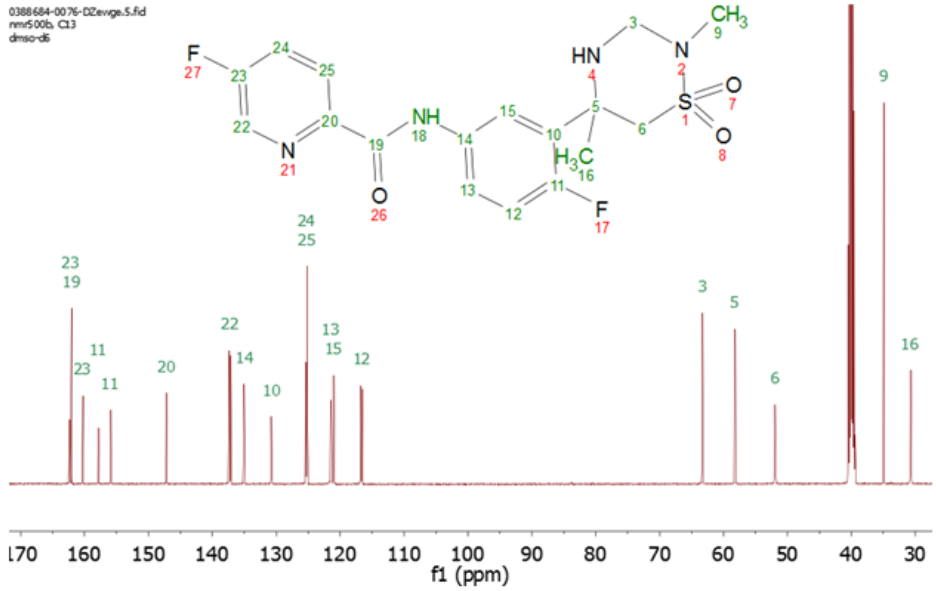
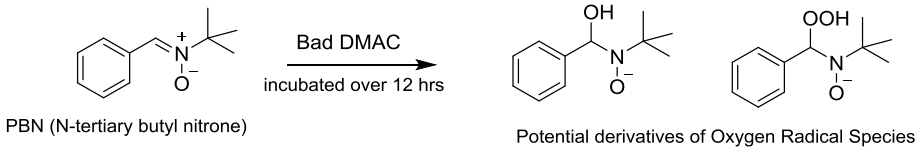


Figure S10:
a) ^{1}H NMR spectrum. b) ^{13}C NMR spectrum of carbon bridged derivative **13**.

17. **EPR Instrumentation/Method:** EPR experiments were conducted on a Bruker EMX instrument at 40^{o}C with microwave frequency/power of 9.4GHz/2mW and modulation amplitude/frequency of 1G/100kHz.



Scheme S3: Use of spin trapping reagent for EPR experiments

18. EPR of air exposed DMAc vs clean DMAc

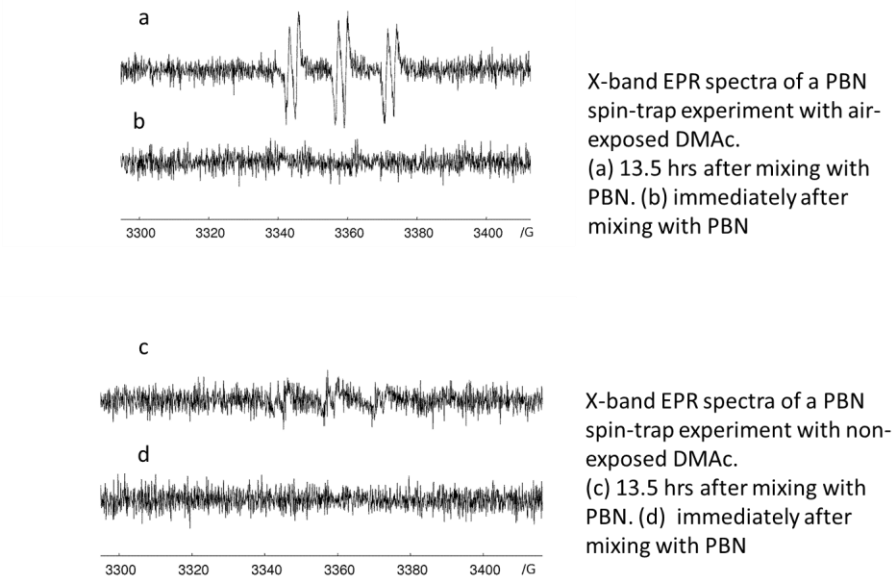


Figure S11: EPR experiment showing Good DMAc sample (a) exposed to air over months (b) kept in closed container over months