# Supporting Information

# Probing the Radical and Base Dual Properties of Peptide Sulfinyl Radicals via Mass Spectrometry

Lei Tan[1], František Tureček[2], Joseph S. Francisco[1], and Yu Xia[1]

[1] Department of Chemistry, Purdue University, West Lafayette, IN, 47907-2084

[2] Department of Chemistry, University of Washington, Seattle, Washington 98195-1700

Address reprint requests to:

Dr. Joseph S. Francisco
Department of Chemistry and Department of Earth and Atmospheric Science
Purdue University
West Lafayette, IN, USA 47907-2084
Phone: (765) 494-7851
Fax: (765) 494-9421
E-mail: jfrancis@purdue.edu

Dr. Yu Xia
Department of Chemistry
Purdue University
West Lafayette, IN, USA 47907-2084
Phone: (765) 494-1142
Fax: (765) 494-9421
E-mail: yxia@purdue.edu

## Materials

*Synthesis of Disulfide Precursor*

The amine was dissolved in water at a concentration of 1.0 mg/mL, and the pH of the solution was adjusted to 8 by adding 0.1 M NaOH. Freshly prepared DSP or $d_8$-DSP (10 mM in DMSO) was then added at a molar ratio of 5:1 (DSP: amine). The mixture was allowed to react at room temperature for 30 min and then subjected to RP-HPLC separation (Agilent 1200 series, Agilent Technologies, Santa Clara, CA). The collected eluent was vacuum dried using a centrivap concentrator (Labconco, Kansas City, MO) and redissolved in water/methanol/acetic acid (v/v/v=50:49:1) at 10 μM for MS analysis. To prepare the sodium-ion adduct, the sample was reconstituted in water/methonal (v/v=1:1) at 10 μM, and 10 mM NaOH was added to adjust the pH to 8. Acetylation was achieved by mixing the tetrapeptide disulfide-linked dimer solution (100 μL, approximately 0.2 mg/mL in water, pH = 5) and 10 μL of acetylation solution (acetic anhydride/methanol (v/v) = 1:3). Notably, the pH of the solution was adjusted to acidic conditions to avoid acetylation at the ε-$NH_2$ from the Lys side chain.[S1]

## Theoretical calculations

*Optimization for $[^{SO\bullet}$Cys-Arg + H$]^+$*

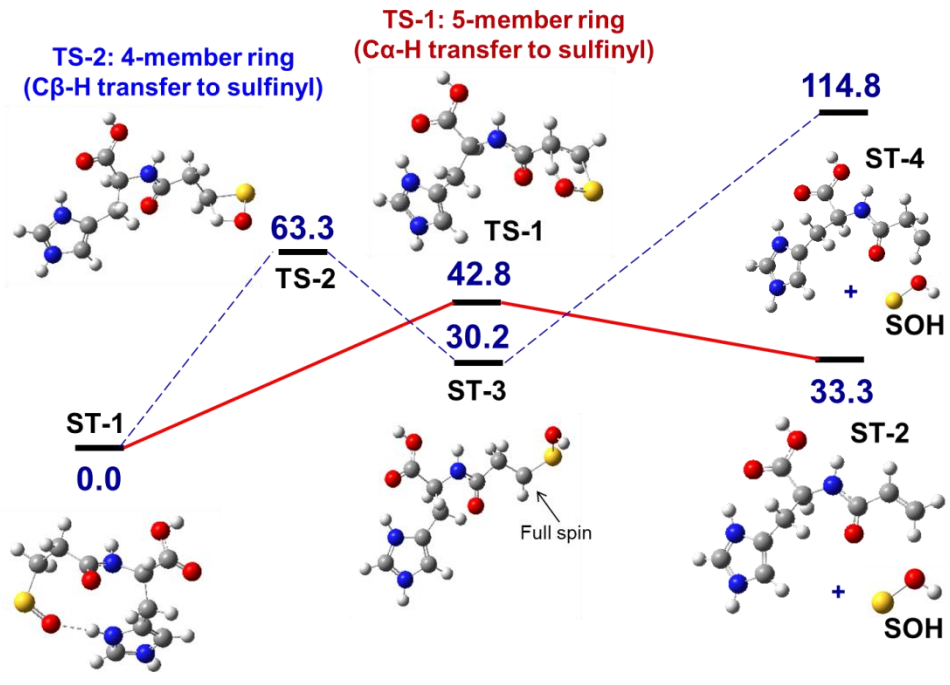
Exhaustive search of the conformational space of the dipeptide sulfinyl radical $[^{SO\bullet}$Cys-Arg + H$]^+$ using the ConformSearch engine[S2] is very challenging because the force fields used in the molecular dynamics step have not been optimized for sulfinyl radicals. Therefore, an indirect approach was adopted, using the [Asn-Arg + H]$^+$ ion protonated at Arg as a proxy of analogous hydrogen bonding properties for the Asn amide and SO groups. Replica exchange molecular dynamics[S3] with CHARMM[S4]/NAMD[S5] produced 800,000 structures from which 8000 were sampled for full geometry optimization with PM6.[S6] Over 100 lowest-energy [Asn-Arg + H]$^+$ structures were selected and converted to sulfinyl radical analogues by deleting the amide group (-$NH_2$) from the Asn side chain and changing the carbonyl C=O to S=O. These cation-radical constructs were then fully optimized with B3LYP[S7] and M06-2X[S8]/6-31+G(d,p), the structures were characterized as local energy minima by harmonic frequency analysis (all frequencies real), and their relative energies were determined by single point calculations with B3LYP, M06-2X and MP2(frozen core)[S9] and the 6-311++G(2d,p) basis set.

*Benchmark Calculation for Proton Affinity*

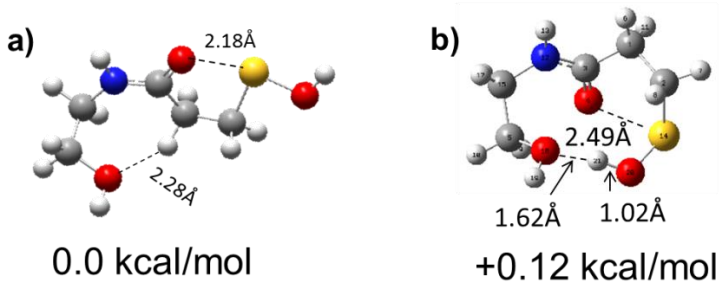
Proton affinity is calculated as the enthalpy difference between protonated sulfinyl radical and the neutral structure. This method has been successfully applied in predicting the proton affinity of small molecules and the results agree well with experimental values.[S10] Three model molecules, methonal, propylamine, and pyridine, were used to calibrate the calculation method. The result is listed in SI Table 1, which yields r.m.s error of 1.6 kcal $mol^{-1}$.

**SI Table 1.** Benchmark calculation of proton affinity by three model compounds. Level of calculation CCSD(T)//B3LYP/6-31G(d) + ΔZPE

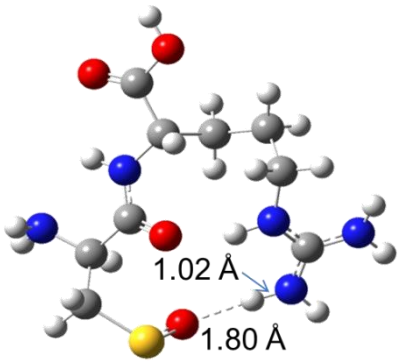
| Name | Calc. (kcal $mol^{-1}$) | Exp. (kcal $mol^{-1}$) [S11] | Δ(Calc.-Exp.) (kcal $mol^{-1}$) |
|---|---|---|---|
| Methanol | 181.0 | 180.3 | 0.7 |
| Propylamine | 221.1 | 219.4 | 1.7 |
| Pyridine | 224.5 | 222.3 | 2.2 |



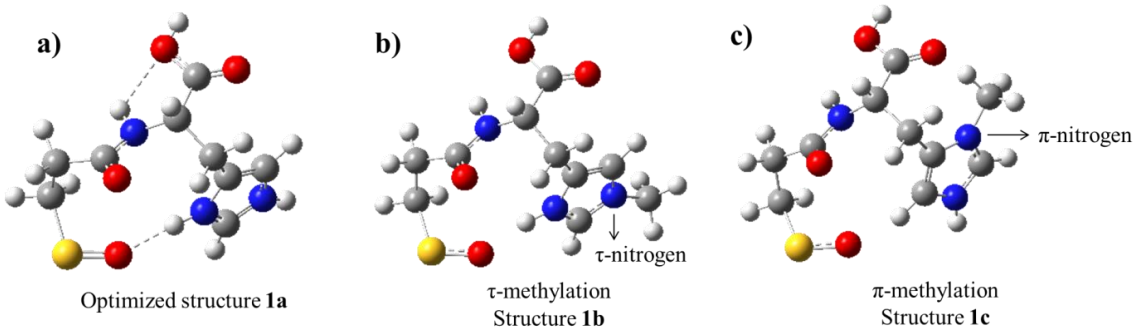

**SI Scheme 1.** Energy diagram for radical induced SOH loss comparing involvement of $C_\alpha$-H vs. $C_\beta$-H, using [n-$^{SO\bullet}$Cys-His + H]$^+$ as the model system. For the mechanism involving the $C_\alpha$-H, the sulfinyl radical oxygen grabs the Cα-H of the cysteine residue through a five-member ring transition state, followed by the leaving of SOH to form a dehydroalanine motif. The transition state and the products are 42.8 kcal mol$^{-1}$ and 33.3 kcal/mol higher in enthalpy relative to the initial structure, which are quite reasonable for low energy CID conditions. However, if $C_\beta$-H were involved, the fragmentation should have gone through a high energy four-member ring transition state (63.3 kcal mol$^{-1}$ higher relative to the initial structure), and the resulting motif from the SOH loss does not obey the octet rule and is thus unstable (product ions with energy of 114.8 kcal mol$^{-1}$ higher relative to the initial structure). It shows that the 5-member ring transition state to abstract $C_\alpha$-H is more favorable than the 4-member ring transition state to abstract the $C_\beta$-H. Level of calculation: CCSD(T)//B3LYP/6-31G(d) + ΔZPE.



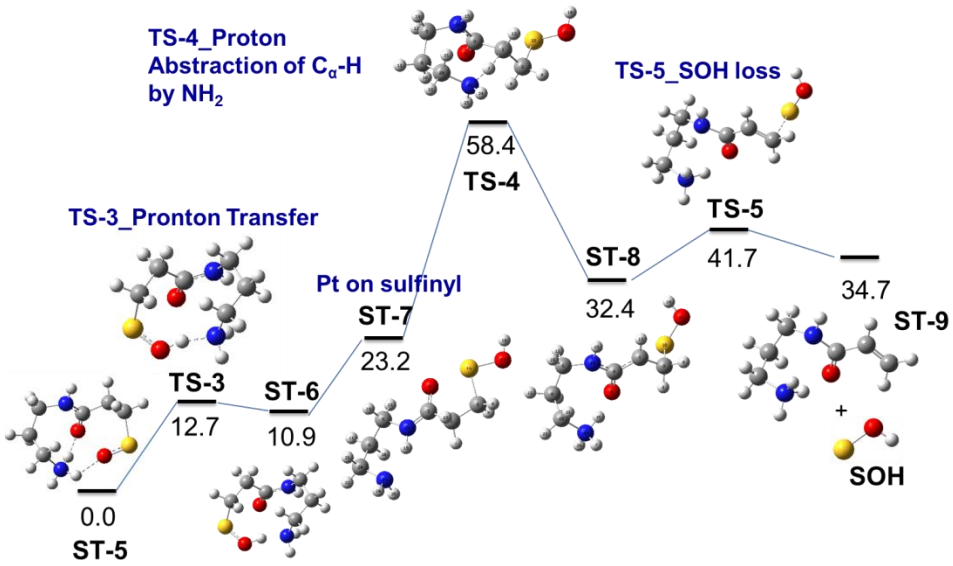

**SI Scheme 2.** Geometry optimization of protonated **4a**. The relative energy of two low energy structures are listed, with the protonation on sulfinyl radical being the most stable one. Level of calculation: B3LYP/6-31G(d) + ΔZPE.



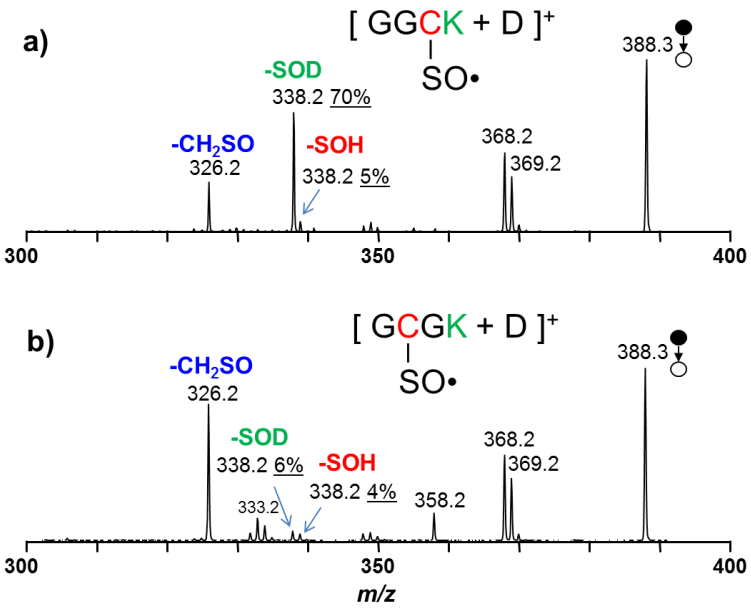

**SI Scheme 3.** Geometry optimization of protonated $^{SO\bullet}$Cys-Arg.




**SI Scheme 4.** 3D structure for compound **1a-c**. Structure **1a** is optimized on level of B3LYP/6-31G(d), while structure **1b** and **1c** are not optimized. It can be seen from the model that the proton bridging between sulfinyl radical and π-nitrogen can be retained in case of τ-methylation (b). However, it is disrupted in π-methylation (scheme c). No matter how we rotate the bonds, it is difficult for the proton on τ-nitrogen to access sulfinyl radical or other backbone atoms.

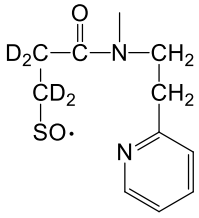
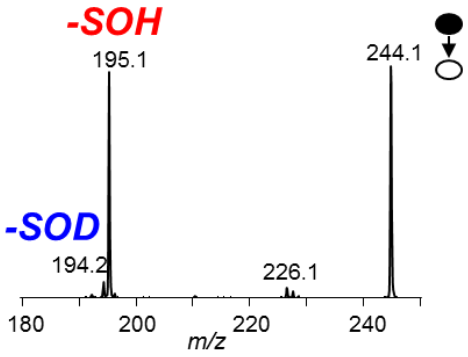
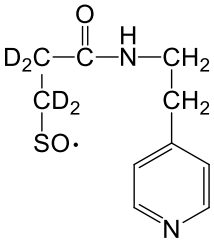
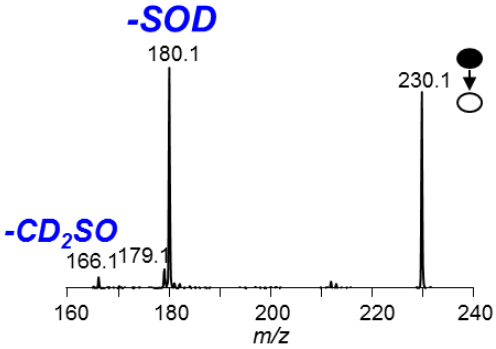
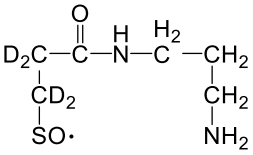
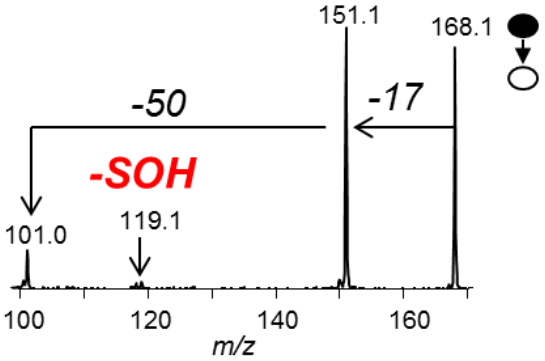


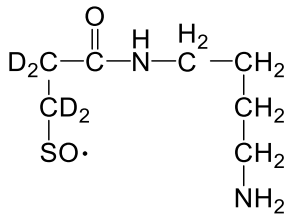

**SI Scheme 5.** Energy diagram for proton induced SOH loss, using protonated **3b** as the model system. Level of calculation: CCSD(T)//B3LYP/6-31G(d) + ΔZPE.



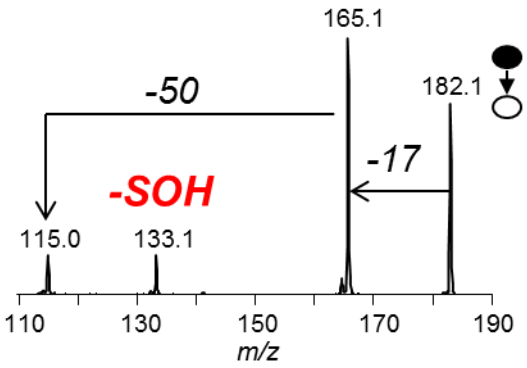

**SI Figure 1.** $MS^2$ ion trap CID of tetrapeptide sulfinyl radical after solution phase hydrogen/deuterium exchange: a) $[GG^{SO\bullet}CK + D]^+$; b) $[G^{SO\bullet}CGK + D]^+$. The degree of deuterium exchange is about 95%.

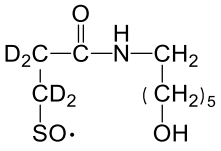
**SI Table 2.** CID spectra of $d_4$- n-$^{SO\bullet}$Cys-X, the number of compound is indicated below each structure.

| Compound (protonated) | CID spectra |
|---|---|
|  **2a** |  |
|  **2c** |  |
|  **3a** |  |

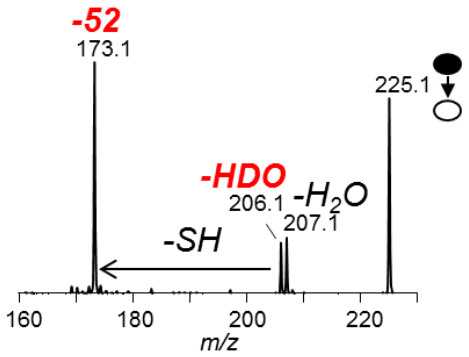


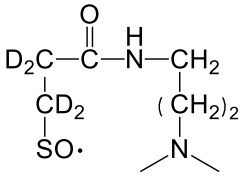
**3b**


165.1
182.1
-50
-17
-SOH
115.0
133.1
110
130
150
170
190
m/z

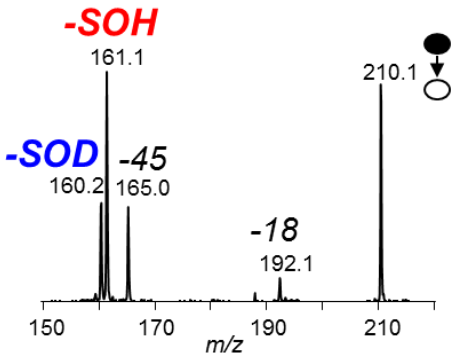


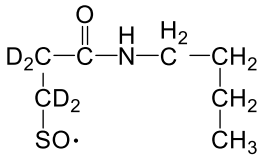
**4b**


-52
173.1
225.1
-HDO
206.1
-H2O
207.1
-SH
160
180
200
220
m/z

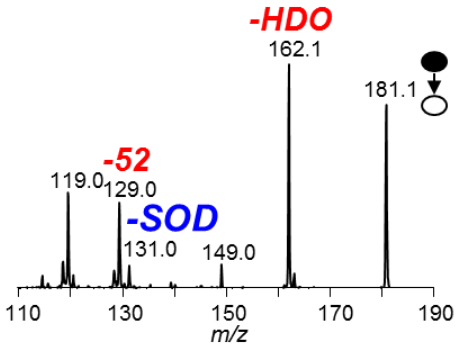


**6**


-SOH
161.1
210.1
-SOD
-45
160.2
165.0
-18
192.1
150
170
190
210
m/z



**7**


-HDO
162.1
181.1
-52
119.0
129.0
-SOD
131.0
149.0
110
130
150
170
190
m/z

**SI Table 3.** Electronic energies (kcal $mol^{-1}$) for key optimized structures. Level of theory: CCSD(T)//B3LYP/6-31G(d)

| Name | CCSD (T) | ΔZPE/B3LYP | CCSD (T) + ΔZPE |
|---|---|---|---|
| **SI Scheme 1** | | | |
| **ST-1** | 0.0 | 0 | 0.0 |
| **ST-2 + SOH** | 36.3 | -3.0 | 33.3 |
| **ST-3** | 32.1 | -1.9 | 30.2 |
| **ST-4 + SOH** | 120.4 | -5.7 | 114.8 |
| **TS-1** | 46.3 | -3.5 | 42.8 |
| **TS-2** | 67.5 | -4.2 | 63.3 |
| **SI Scheme 2** | | | |
| **ST-5** | 0.0 | 0.0 | 0.0 |
| **ST-6** | 11.4 | -0.5 | 10.9 |
| **ST-7** | 24.9 | -1.7 | 23.2 |
| **ST-8** | 34.5 | -2.1 | 32.4 |
| **ST-9 + SOH** | 38.2 | -3.5 | 34.7 |
| **TS-3** | 13.5 | -0.8 | 12.7 |
| **TS-4** | 63.6 | -5.2 | 58.4 |
| **TS-5** | 44.4 | -2.7 | 41.7 |

**Gaussian archive entries for B3LYP/6-31G(d) calculation for results shown in SI Scheme 1 and 4**

## ST-1

1\1\GINC-CARTER-A076\FOpt\UB3LYP\6-31G(d)\C9H13N3O4S1(1+,2)\FRANCISC\2
6-Aug-2013\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=calcfc\\nCH 25
9 sulfinyl, proton bridging between pi-N and sulfinyl_2\\1,2\C,0.26998
80697,-0.0461315607,-2.0093475802\C,0.8368339503,-0.0472550568,-3.4446
443598\C,-0.4291211469,1.2612409322,-1.6492249062\O,0.2099127787,2.249
5437835,-1.3106018597\C,-2.5651133638,2.4276272038,-1.2993547713\C,-4.
0592172401,2.104422737,-1.2984179169\O,-4.9305169065,2.914517666,-1.51
27127335\H,-0.7017972211,3.0159127658,-4.30777813\C,-2.2921129548,3.75
08204647,-2.0522074792\H,-0.3969059664,-0.9110967997,-1.9108933677\H,1
.4674706301,-0.9311601079,-3.594212028\H,0.039919024,-0.067304986,-4.1
95730945\H,-1.2419563596,4.0046837216,-1.8947353368\H,-2.9026762617,4.

5255260614,-1.5828975247\H,-2.2851338924,2.6148959599,-0.2509948337\H,
1.0841693304,-0.1703515893,-1.2900149454\C,-2.5960912792,3.729817304,-
3.5161471732\C,-3.7293585129,4.0779953391,-4.2025062512\H,-4.68628438,
4.4195225128,-3.8415162845\N,-1.6704571465,3.3498991052,-4.479111494\N
,-3.4554791516,3.9079794741,-5.5470234953\H,-4.0946234812,4.0998628907
,-6.3090614962\C,-2.1998417333,3.4669149893,-5.6951427147\H,-1.7040033
062,3.2525808884,-6.6293374449\O,-4.2943000592,0.8103742392,-0.9993815
917\H,-5.2629310767,0.6826710557,-0.9719248823\N,-1.7948376964,1.28805
947,-1.7996633925\H,-2.2893898355,0.4056441913,-1.8426910195\S,1.89665
74706,1.3924464799,-3.8792641446\O,0.9405227081,2.4850554753,-4.381488
4074\\Version=EM64L-G09RevC.01\State=2-A\HF=-1213.902492\S2=0.753663\S
2-1=0.\S2A=0.75001\RMSD=6.635e-09\RMSF=3.573e-06\Dipole=-1.7773178,-0.
6073516,-2.3764812\Quadrupole=-0.6091796,-2.8259717,3.4351513,-11.0762
179,4.1018037,-10.9179718\PG=C01 [X(C9H13N3O4S1)]\\@

## ST-2

1\1\GINC-CARTER-A256\FOpt\RB3LYP\6-31G(d)\C9H12N3O3(1+)\FRANCISC\26-Au
g-2013\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=calcfc\\nCH 210, d
ehydroalanine, geo opt\\1,1\C,0.7270558742,0.6602030776,-0.0969798541\
C,1.1357965451,-0.5492209853,0.2949054467\C,-0.6817891846,0.8843363903
,-0.5046386105\O,-1.560455302,0.02401927,-0.4895018191\C,-2.2952356954
,2.4276322482,-1.4586812274\C,-2.3256216027,3.687027594,-2.3166109174\
O,-3.3538630828,4.2482169731,-2.6637643699\H,-4.9928325445,3.546721724
5,-2.2941451889\C,-3.3761723977,2.4774781716,-0.3318578753\H,0.4389836
909,-1.3819189565,0.3221450326\H,2.1627703707,-0.7357074837,0.59209233
92\H,-3.3719943425,3.4635174189,0.147256083\H,-3.0704167534,1.74209699
07,0.4128304268\H,-2.5542898066,1.5879089723,-2.1223460764\H,1.4124265
855,1.5048060922,-0.129837068\C,-4.7637656011,2.1179531499,-0.76343992
34\C,-5.6148003785,1.1408974205,-0.3219520354\H,-5.4860287169,0.382460
2713,0.43424792\N,-5.4536055663,2.7979864735,-1.7568933318\N,-6.782769
321,1.2631745265,-1.0538579655\H,-7.6051356981,0.6792128292,-0.9550882
378\C,-6.6651315357,2.2723500638,-1.9252878114\H,-7.4134429757,2.59372
02288,-2.6335406516\O,-1.1177416349,4.1200206575,-2.6785050572\H,-1.22
94255537,4.9130643705,-3.2405236626\N,-0.9838973285,2.1727338631,-0.90
46238199\H,-0.2462726639,2.8442906078,-1.0671069445\\Version=EM64L-G09
RevC.01\State=1-A\HF=-739.9036449\RMSD=8.860e-09\RMSF=2.299e-06\Dipole
=-3.0939386,0.7690452,-0.4897806\Quadrupole=30.9715834,-14.4905492,-16
.4810342,6.3068852,3.6128835,-7.256539\PG=C01 [X(C9H12N3O3)]\\@

## ST-3

1\1\GINC-CARTER-A183\FOpt\UB3LYP\6-31G(d)\C9H13N3O4S1(1+,2)\FRANCISC\2

6-Aug-2013\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=calcfc\\nCH --
-> hoS loss ( 4 center transition state, grab belta-carbon hydrogen) h
ydrogen transfer to so group\\1,2\C,2.8025108381,0.4720746926,-0.59627
93389\C,3.6918074206,-0.729834587,-0.6757038988\C,1.3273089654,0.09915
73973,-0.5804627577\O,0.8660015987,-0.9087634129,-1.1035080162\C,-0.93
60404637,0.8211359962,-0.0692743305\C,-1.6995123634,2.112514304,0.2032
546012\O,-2.9004211431,2.1576772149,0.4221612736\H,-3.9523096812,0.670
6938087,0.3149775509\C,-1.4400070872,-0.3436690429,0.840976225\H,3.064
1328732,1.08278406,0.2759370134\H,5.3265773358,-0.3079585048,1.8624926
509\H,3.3556224708,-1.6088962841,-1.2166946959\H,-1.5596233108,0.01141
26181,1.8712565274\H,-0.6501464324,-1.0954952938,0.8361414117\H,-1.154
5526582,0.5402267377,-1.1110488807\H,2.9494846284,1.1247843678,-1.4776
393813\C,-2.6941590382,-1.0181444765,0.3785049631\C,-2.9438376253,-2.3
267059229,0.0637314005\H,-2.2931811441,-3.1871296177,0.0570770685\N,-3
.8985614772,-0.3517799438,0.2023337025\N,-4.2802236014,-2.4091718808,-
0.2856348424\H,-4.765545575,-3.2528361456,-0.5682497214\C,-4.843863246
4,-1.1981423096,-0.2008531073\H,-5.871932043,-0.9562330551,-0.42336826
8\O,-0.9369254361,3.2049739199,0.1633012682\H,-1.5042632672,3.98507929
41,0.327465896\N,0.4960324259,0.9951115948,0.055733691\H,0.8561592703,
1.8803553101,0.3851048893\S,5.3586823697,-0.658064285,-0.3013237471\O,
5.3936429957,0.306675057,1.1090979006\\Version=EM64L-G09RevC.01\State=
2-A\HF=-1213.8560979\S2=0.759568\S2-1=0.\S2A=0.750041\RMSD=9.578e-09\R
MSF=3.303e-06\Dipole=-5.9393354,-0.9402591,0.5574768\Quadrupole=26.386
1553,-1.4626553,-24.9235,15.2983966,6.4658233,0.5761281\PG=C01 [X(C9H1
3N3O4S1)]\\@

## ST-4

1\1\GINC-CARTER-A076\FOpt\RB3LYP\6-31G(d)\C9H12N3O3(1+)\FRANCISC\26-Au
g-2013\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=calcfc\\nCH 210, a
fter grabbing belta-carbon hydrogen\\1,1\C,1.0941009481,0.7254131536,-
0.522846121\C,1.3474347493,-0.2709498166,0.5269450014\C,-0.3140633731,
0.898167436,-1.1018695232\O,-1.0724461138,-0.0389131079,-1.3188440045\
C,-1.9918762088,2.4416256341,-1.9466816808\C,-2.071463091,3.8353653542
,-2.5614303365\O,-3.1161683409,4.3658809149,-2.9051075962\H,-4.7137515
547,3.4882765068,-2.8265128056\C,-3.1625088562,2.1911805589,-0.9433499
885\H,1.6372169228,1.670836329,-0.3942242254\H,0.520647544,-1.00915557
06,0.4310541595\H,-3.2890635809,3.064000626,-0.2921589078\H,-2.8544054
975,1.3519052641,-0.3189040494\H,-2.1073807471,1.7246392894,-2.7735267
24\H,1.6836132168,0.1474281197,-1.2807188948\C,-4.4719300545,1.8166053
727,-1.5662856364\C,-5.2627787777,0.7133131708,-1.389573627\H,-5.12587
324,-0.1617001136,-0.7735820657\N,-5.1421523446,2.6186313025,-2.478996
2629\N,-6.3772587665,0.8840694056,-2.1914740271\H,-7.1491334446,0.2325

477949,-2.2758665773\C,-6.2848753491,2.0452772763,-2.8505327377\H,-7.0
026651016,2.4379332858,-3.5545512909\O,-0.884883629,4.4244819172,-2.70
74777466\H,-1.0218764453,5.3002818294,-3.1219423645\N,-0.7083726297,2.
1952230547,-1.321081554\H,-0.0463525744,2.9546919418,-1.2395921834\\Ve
rsion=EM64L-G09RevC.01\State=1-A\HF=-739.7705587\RMSD=6.753e-09\RMSF=2
.105e-06\Dipole=-3.8887943,0.9762216,-1.4527081\Quadrupole=23.6772644,
-8.0854501,-15.5918144,14.969583,1.4588777,-1.198658\PG=C01 [X(C9H12N3
O3)]\\@

## ST-5

1\1\GINC-CARTER-A314\FOpt\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\FRANCISC\2
9-Jan-2014\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=readfc geom=ch
eck guess=read\\h+nh2(ch2)3nhc(o)ch2ch2so radical cation protonation a
t nh2 site Lei-Lei structure\\1,2\C,1.3883048434,0.7266371198,0.332502
6053\C,2.6435992351,0.8178356447,-0.5621105398\C,0.243934683,1.6468788
368,-0.0873479831\O,0.0444257216,2.7151921161,0.5131030985\C,-1.844320
9215,3.3715319225,-1.7294817588\H,1.0567265834,-0.31932814,0.332050271
7\H,3.4273747184,0.1626287472,-0.1648558786\H,2.4389736396,0.514085157
5,-1.5944578235\H,-2.0702749881,3.785382476,-0.7421775926\H,-2.7214014
37,3.5815929211,-2.3505551821\H,1.6378814915,0.9935931435,1.3621360512
\N,-0.4890011677,1.2484250623,-1.1514264119\H,-0.2794456726,0.33135176
1,-1.5257696416\S,3.4098167495,2.4846439639,-0.656984389\C,-1.74743170
79,1.8349125499,-1.6428616741\H,-2.579502095,1.4808840269,-1.022347255
7\H,-1.8898044051,1.4132381725,-2.6421348424\O,2.4766966114,3.30405237
7,-1.5637392814\H,0.1555428004,4.1036670113,-0.3936320571\C,-0.6270240
333,4.0967996669,-2.330125281\H,0.0483121807,3.4129902523,-2.84702373\
H,-0.9181169375,4.8878226964,-3.0229275449\N,0.196980236,4.739552446,-
1.2348443197\H,1.1933473064,4.7870915305,-1.4923095043\H,-0.1488719247
,5.6686812378,-0.981580385\\Version=EM64L-G09RevC.01\State=2-A\HF=-894
.9687223\S2=0.753711\S2-1=0.\S2A=0.75001\RMSD=8.214e-09\RMSF=7.673e-06
\Dipole=-0.944255,0.3727687,-0.8554475\Quadrupole=1.2161249,9.6259462,
-10.8420711,-6.3990633,6.9792141,-3.3752338\PG=C01 [X(C6H14N2O2S1)]\\@

## ST-6

1\1\GINC-CARTER-A490\FOpt\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\FRANCISC\1
4-Feb-2014\0\\#p b3lyp/6-31g* scfcyc=300 opt=readfc geom=check guess=r
ead\\h+-nh2(ch2)3ch2nhc(o)ch2ch2so radical cation, minimum structure f
ollowing transfer yupa3.lnh2tsqst folded\\1,2\C,-1.0002512445,-1.62744
9272,-1.0908772012\C,-1.4311376725,-0.304341141,-1.7586514865\S,-2.409
6560002,0.8171568356,-0.6833110955\H,-0.2788048215,-2.1220068185,-1.75
23621453\C,-0.4616268869,-1.49280506,0.3345289256\O,-1.1635485787,-1.7

605679404,1.2923571326\N,0.826503685,-1.0057366671,0.5396075109\H,1.11
81677545,-1.210934747,1.4927053301\H,-1.8661689065,-2.2862737475,-0.99
94984547\H,-0.5800531433,0.2924551496,-2.1046556548\H,-2.0639229742,-0
.5146054271,-2.628375498\O,-1.3628357727,1.5523679321,0.1741359028\C,1
.9108868345,-0.8950824881,-0.4348163479\C,2.8832431826,0.2277861253,-0
.0437337981\H,1.4826558583,-0.6863399173,-1.4194073273\H,2.4749371171,
-1.833580686,-0.5228218157\H,3.2952860918,0.0333660522,0.9568432532\H,
3.7421348184,0.2003255616,-0.7246865087\C,2.3361527334,1.6554142736,-0
.1049085045\H,1.9185739128,1.8934978736,-1.0869639835\H,3.1300394212,2
.3736640431,0.1123451831\H,1.0573219828,2.9321913134,0.9924417928\H,0.
3037977555,1.5074319403,0.5967397725\N,1.2239911218,1.9244595736,0.891
8533691\H,1.4500653193,1.5573637452,1.8216879984\\Version=EM64L-G09Rev
C.01\State=2-A\HF=-894.9492718\S2=0.753688\S2-1=0.\S2A=0.75001\RMSD=9.
582e-09\RMSF=2.753e-05\Dipole=2.7326225,1.8191357,-0.5136184\Quadrupol
e=5.1501283,-0.5746264,-4.575502,5.6129247,9.7259151,7.7972654\PG=C01
[X(C6H14N2O2S1)]\\@

## ST-7

1\1\GINC-CARTER-A269\FOpt\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\ROOT\04-Fe
b-2014\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=readfc geom=check
guess=read\\nh2(ch2)3nhc(o)ch2ch2so-h+ radical cation protonation at s
-o site Structure before grabbing alpha-carbon hydrogen with amine, pt
at sulfinyl\\1,2\C,-2.9187486804,2.0676305069,2.3069342889\C,-3.22477
20888,0.5865632519,2.5051744926\C,-2.8212300522,2.344123177,0.81679343
56\O,-2.4708152184,1.3676027714,0.0828865257\C,-2.9161744179,3.9175902
529,-1.0761859149\C,-3.7975570195,5.1309455765,-1.3969984986\H,-3.6858
178925,2.6808928489,2.788513559\H,-2.9215308068,0.2136828022,3.4860919
664\H,-4.2850324319,0.3556804159,2.3567888741\H,-4.8562865172,4.857124
8296,-1.2951985334\H,-3.6426393814,5.3838270156,-2.4518169203\H,-3.186
7727394,3.0526798488,-1.6874168287\H,-1.9554412472,2.3272769906,2.7636
616568\N,-3.0520466167,3.5474151292,0.343708735\H,-3.3121824584,4.3522
755189,0.9535154797\S,-2.3217606188,-0.4289626356,1.2661221561\O,-2.85
05810797,-1.8439218528,1.9965728096\H,-2.4924211149,-2.6095271706,1.50
24763339\H,-1.8620089209,4.1466267562,-1.2787388956\C,-3.4973974259,6.
3695826261,-0.5404762824\H,-2.4247849898,6.5930100144,-0.5790646881\H,
-4.0207631587,7.2406415604,-0.957201519\N,-3.8512498271,6.1257039874,0
.8818870097\H,-3.4483620657,6.8534905922,1.47081731\H,-4.8622395798,6.
1992311862,0.9991425781\\Version=EM64L-G09RevC.01\State=2-A\HF=-894.93
66179\S2=0.753621\S2-1=0.\S2A=0.750011\RMSD=6.216e-09\RMSF=7.085e-06\D
ipole=-0.170326,-0.1883176,0.9016281\Quadrupole=-15.0242755,24.9287905
,-9.904515,-7.620105,1.0519533,-7.1962102\PG=C01 [X(C6H14N2O2S1)]\\@

## ST-8

1\1\GINC-CARTER-A192\FOpt\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\ROOT\04-Fe
b-2014\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=readfc geom=check
guess=read\\nh2(ch2)3nhc(o)ch2ch2so-h+ radical cation protonation at s
-o site Structure after grabbing alpha-carbon hydrogen with amine\\1,2
\C,-2.3972476315,1.3888257263,1.897305464\C,-3.3475934624,0.6196047137
,2.7085265228\C,-2.8603940241,2.3929527799,0.9732358198\O,-4.092162915
9,2.6538879036,0.8487333782\C,-2.0773315377,3.7509060444,-1.0301286405
\C,-3.2832808554,4.6871979034,-1.2300334048\H,-4.600175595,5.661716347
,1.7304677947\H,-2.957260469,0.2791137223,3.6696248352\H,-4.301536908,
1.1344190076,2.8430450735\H,-4.1906941596,4.1003636958,-1.4050590361\H
,-3.0920860627,5.231827182,-2.1606414292\H,-2.1150261247,2.9909316196,
-1.822072651\H,-1.3466500793,1.1121572508,1.9100896139\N,-1.9098749487
,3.0946517861,0.2773823813\H,-0.9551917705,2.8035377547,0.4522818111\S
,-3.762330791,-0.9248530791,1.7387648367\O,-2.5179077817,-1.9301455701
,2.2498475226\H,-1.9264620362,-2.0783749875,1.4911665347\H,-1.15921369
7,4.3263683917,-1.1824500425\C,-3.5370282942,5.7106320133,-0.111744787
\H,-2.6459523509,5.8815223351,0.4966859516\H,-3.8778930863,6.671047306
5,-0.5027861406\N,-4.6080251025,5.1916455651,0.8211112124\H,-5.5413335
06,5.3178148674,0.4178049625\H,-4.4499969695,4.1149661004,0.9387717767
\\Version=EM64L-G09RevC.01\State=2-A\HF=-894.9235213\S2=0.755789\S2-1=
0.\S2A=0.750022\RMSD=5.355e-09\RMSF=1.913e-05\Dipole=0.1457089,3.81990
32,-0.823492\Quadrupole=-5.278813,15.5752128,-10.2963998,-12.709051,-2
.0903397,-3.4080688\PG=C01 [X(C6H14N2O2S1)]\\@

## ST-9

1\1\GINC-CARTER-A505\FOpt\RB3LYP\6-31G(d)\C6H13N2O1(1+)\ROOT\12-Feb-20
14\0\\#p b3lyp/6-31g* scfcyc=300 opt=readfc geom=check guess=read\\str
ucture following soh loss from yupa3 structure Lei-Lei structure\\1,1\
C,-2.3921786599,1.4325675794,1.989975034\C,-3.2460507297,0.7886578568,
2.7922628222\C,-2.8790594234,2.4208888452,1.002607437\O,-4.0945918347,
2.7116033343,0.9062815437\C,-2.0779600624,3.7365370245,-1.0334598905\C
,-3.2910693664,4.6643281993,-1.2153197815\H,-4.6570197417,5.7580915956
,1.6930616042\H,-2.9019762128,0.0487571423,3.5076147445\H,-4.312796564
1,0.9842379342,2.7394624152\H,-4.1984156029,4.0720021295,-1.3683816687
\H,-3.1155746831,5.1942039804,-2.1574815011\H,-2.0935693432,3.01251638
23,-1.8579799876\H,-1.3244281533,1.2289202943,2.029188238\N,-1.9247539
992,3.0085959061,0.2377738705\H,-0.9807349272,2.6757554529,0.395283316
3\H,-1.1627787835,4.3267571789,-1.1451789169\C,-3.5284712298,5.7099544
298,-0.1115464578\H,-2.6460862236,5.8536744312,0.5166218047\H,-3.82093
47448,6.6790440939,-0.5196787989\N,-4.6410945678,5.2498025489,0.804182
0268\H,-5.5580556955,5.370261202,0.362782723\H,-4.4983490009,4.1889324
782,0.972079873\\Version=EM64L-G09RevC.01\State=1-A\HF=-420.9664378\RM

SD=4.164e-09\RMSF=4.529e-06\Dipole=-0.2963946,2.0198718,-0.0097212\Qua
drupole=-1.1394991,4.9463867,-3.8068876,-9.5434703,-1.9155284,-5.13413
25\PG=C01 [X(C6H13N2O1)]\\@

## TS-1

1\1\GINC-CARTER-A213\FTS\UB3LYP\6-31G(d)\C9H13N3O4S1(1+,2)\FRANCISC\27
-Aug-2013\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=(ts,calcfc,noei
gen) optcyc=100\\nCH ---> hoS loss ( 5 center transition state )\\1,2\
C,0.7747058658,0.7459522578,0.0786425915\C,1.2519458043,-0.5736240297,
0.1935881632\C,-0.6107327193,0.9334901898,-0.3931111095\O,-1.473106759
7,0.0522494083,-0.388651134\C,-2.2495181818,2.4199943047,-1.3955006014
\C,-2.2927999464,3.6563016006,-2.2855463873\O,-3.3246081409,4.20234860
61,-2.6478436746\H,-4.9532429933,3.4985454955,-2.2784589122\C,-3.34911
67565,2.4703921017,-0.287543739\H,0.5519296134,0.9405405471,1.57460218
6\H,0.5928657987,-1.3890218706,-0.0884587102\H,2.3151988952,-0.7749338
828,0.1127482929\H,-3.3618656934,3.4616548377,0.1806171609\H,-3.043613
6488,1.7467274182,0.4685842622\H,-2.4785372426,1.5587526308,-2.0429782
5\H,1.493311874,1.5460649016,-0.0971689371\C,-4.7277556804,2.090654842
1,-0.7298339128\C,-5.5744361674,1.1113469135,-0.2845499337\H,-5.446391
799,0.3637403965,0.4824225644\N,-5.4148646747,2.7530276091,-1.73679380
41\N,-6.7371260497,1.215103397,-1.0278681886\H,-7.5556431354,0.6259545
455,-0.9290034237\C,-6.6202877254,2.2153371591,-1.909817495\H,-7.36485
60354,2.5220553007,-2.6283817189\O,-1.0890739896,4.088390098,-2.663704
5427\H,-1.2105166112,4.8648691892,-3.2463051011\N,-0.9445116306,2.2150
578253,-0.8077767601\H,-0.2156032427,2.8865596397,-1.0051386544\S,1.08
80183661,-0.9168116516,2.514348091\O,0.5475400869,0.566096789,2.646746
4584\\Version=EM64L-G09RevC.01\State=2-A\HF=-1213.8427471\S2=0.754229\
S2-1=0.\S2A=0.750014\RMSD=7.717e-09\RMSF=7.203e-06\Dipole=-3.9927632,1
.0095485,-1.8926168\Quadrupole=37.6145405,-15.5072926,-22.1072479,-1.5
414438,9.9311982,-10.6732013\PG=C01 [X(C9H13N3O4S1)]\\@

## TS-2

1\1\GINC-CARTER-A233\FTS\UB3LYP\6-31G(d)\C9H13N3O4S1(1+,2)\FRANCISC\26
-Aug-2013\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=(ts,calcfc,noei
gen)\\nCH ---> hoS loss ( 4 center transition state, grab belta-carbon
hydrogen)\\1,2\C,0.6396154534,-0.2028306644,-0.4130676148\C,1.3212251
692,0.5937307119,0.6881987799\C,-0.7470519091,0.308188546,-0.808063520
1\O,-1.5484539369,-0.3771773559,-1.4338561284\C,-2.2630126302,2.197763
118,-0.9479987225\C,-2.2139548735,3.7208509317,-0.886032392\O,-3.19813
73618,4.4376640155,-0.9916845995\H,-4.8382916669,3.7574969749,-1.37332
58454\C,-3.5186311486,1.6339864745,-0.2092199146\H,1.2373609111,-0.177

1088544,-1.3344155431\H,1.8669882639,2.0407347576,0.8251059059\H,0.896
2277948,0.4151618814,1.6833755254\H,-3.6424283756,2.1389482652,0.75596
18737\H,-3.3066674632,0.5823880276,-0.0132501359\H,-2.362229145,1.9291
483614,-2.0112895394\H,0.4997811419,-1.2636013571,-0.170257222\C,-4.79
6283702,1.687523782,-0.9876325822\C,-5.6601600604,0.6934329717,-1.3611
362466\H,-5.6214391736,-0.3724169053,-1.1993409845\N,-5.3458268481,2.8
659664711,-1.4718469227\N,-6.6966285444,1.2982713083,-2.0501591935\H,-
7.4950250241,0.8216396743,-2.4532124825\C,-6.48624637,2.618747457,-2.1
125495053\H,-7.1224827204,3.3436067991,-2.5969936925\O,-0.9859476164,4
.2111133189,-0.7243970134\H,-1.0410694397,5.1879389476,-0.7160752115\N
,-1.0499871836,1.5948483402,-0.4440325289\H,-0.3274313632,2.1633279061
,-0.0185393495\S,3.088754938,0.5722378738,0.8035378267\O,3.046172014,2
.1743133113,1.0510207797\\Version=EM64L-G09RevC.01\State=2-A\HF=-1213.
8037362\S2=0.753438\S2-1=0.\S2A=0.750008\RMSD=8.772e-09\RMSF=5.411e-06
\Dipole=-5.1836165,0.7741064,-1.1820774\Quadrupole=37.6017893,-16.1186
546,-21.4831347,-5.0346828,19.263363,-5.3365828\PG=C01 [X(C9H13N3O4S1)
]\\@

## TS-3

1\1\GINC-CARTER-A018\FTS\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\ROOT\13-Feb
-2014\0\\#p b3lyp/6-31g* opt=(ts,readfc,noeigen) geom=check guess=read
\\h+-nh2(ch2)3ch2nhc(o)ch2ch2so radical cation, ts to transfer proton
from amine to sulfinyl\\1,2\C,-0.9172476677,-1.4322970651,-1.005315241
9\C,-1.4001775446,-0.1649199208,-1.7431336369\S,-2.4195084365,1.013705
2376,-0.7639473633\H,-0.2249273494,-1.9666181642,-1.6636460805\C,-0.33
93011847,-1.2017025791,0.3896655299\O,-1.0792331044,-0.9857076067,1.34
01641621\N,1.0289678191,-1.2575115753,0.5883929345\H,1.2758240184,-1.4
266658829,1.5599816228\H,-1.7736184563,-2.0918921166,-0.8361570717\H,-
0.5696058539,0.4312864932,-2.137495951\H,-2.0264057842,-0.4578589423,-
2.5935458268\O,-1.4143726858,1.9693545269,-0.0991596235\C,2.1250771712
,-1.0874216963,-0.361431897\C,2.9003284738,0.2235035324,-0.1125869759\
H,1.7134001992,-1.0888693281,-1.3743187683\H,2.8166282394,-1.934427144
7,-0.2957082772\H,3.4017307991,0.1862688036,0.8640178903\H,3.701514662
3,0.3010633053,-0.8570962936\C,2.0624153187,1.501036942,-0.2201991178\
H,1.4589855052,1.5081862045,-1.1313317943\H,2.6963665068,2.3890802853,
-0.2344108595\H,1.3840069469,2.3909730117,1.6041051485\H,0.1215888944,
1.9327243708,0.5676420798\N,1.0785572334,1.6809859453,0.9325053623\H,0
.9347578676,0.7954798706,1.4331863984\\Version=EM64L-G09RevC.01\State=
2-A\HF=-894.9471039\S2=0.75365\S2-1=0.\S2A=0.75001\RMSD=9.337e-09\RMSF
=4.476e-06\Dipole=2.523962,0.6893604,-0.3308093\Quadrupole=5.3650241,-
2.0349943,-3.3300298,4.3215244,9.1140359,5.6592333\PG=C01 [X(C6H14N2O2
S1)]\\@

## TS-4

1\1\GINC-CARTER-A220\FTS\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\FRANCISC\04
-Feb-2014\0\\#p b3lyp/6-31g* scfcyc=300 trans=iabc opt=(ts,readfc,noei
gen) geom=check guess=read\\TS to grab alpha-carbon hydrogen with amin
e\\1,2\C,-2.9668014707,2.1254176952,1.5701888385\C,-3.9739272246,1.124
4531787,2.051602057\C,-2.8269878316,2.2227335516,0.0798503164\O,-3.670
429064,1.7302858269,-0.6650143946\C,-2.1704915098,4.0119778299,-1.4464
590358\C,-3.4185892687,4.8721202751,-1.1407379358\H,-3.5595584714,3.40
0093001,1.5729049931\H,-4.1598961414,1.1318759107,3.1273553725\H,-4.90
64473572,1.1588273388,1.4869933416\H,-4.321968556,4.2741474286,-1.3110
859458\H,-3.4571610854,5.6936146306,-1.8644309944\H,-2.3327203056,3.44
04897202,-2.3627246128\H,-2.054479085,2.1985494861,2.16706496\N,-1.859
9165007,3.0940222844,-0.3475356672\H,-1.2183037098,3.4396777201,0.3550
261618\S,-3.1901934585,-0.4917747303,1.6546133625\O,-2.6228948754,-0.8
647761347,3.1693590561\H,-1.7647865501,-1.3226298534,3.076107835\H,-1.
2906719529,4.6427643779,-1.5993803869\C,-3.4672842491,5.4827245553,0.2
731058075\H,-2.4678111982,5.7149751769,0.6537948467\H,-4.0353022275,6.
4186594438,0.2556603049\N,-4.1269650626,4.5940456884,1.2993216745\H,-4
.20514697,5.0837850308,2.1942476752\H,-5.0787170036,4.3624813575,1.001
1453298\\Version=EM64L-G09RevC.01\State=2-A\HF=-894.8811024\S2=0.75433
7\S2-1=0.\S2A=0.750014\RMSD=3.940e-09\RMSF=6.333e-06\Dipole=0.1214968,
1.6872558,1.5680794\Quadrupole=-6.3882214,15.3124311,-8.9242097,-7.463
0333,-0.5499465,-8.3621939\PG=C01 [X(C6H14N2O2S1)]\\@

## TS-5

1\1\GINC-CARTER-A472\FTS\UB3LYP\6-31G(d)\C6H14N2O2S1(1+,2)\FRANCISC\13
-Feb-2014\0\\#p b3lyp/6-31g* scfcyc=300 opt=(ts,readfc,noeigen) geom=c
heck guess=read\\nh2(ch2)3nhc(o)ch2ch2so-h+ radical cation protonation
at s-o site Structure after grabbing alpha-carbon hydrogen with amine
breaking the C-S bond\\1,2\C,-2.3028065316,1.6236053622,2.0080052821\
C,-3.1718474181,0.9366972041,2.8225904747\C,-2.8002765333,2.6112116706
,1.0585363727\O,-4.0347685408,2.8245494263,0.9133770921\C,-2.032335290
3,4.0150179161,-0.9224727113\C,-3.2691903196,4.9098929747,-1.122125458
2\H,-4.6990515248,5.7940519518,1.8171910694\H,-2.7969033066,0.29235556
25,3.611090758\H,-4.2014306682,1.265604535,2.909533096\H,-4.150849745,
4.2932990247,-1.3245618181\H,-3.082810335,5.4792336045,-2.0388147814\H
,-2.0248098893,3.2776219024,-1.7358340654\H,-1.241672287,1.3891809651,
2.0136298602\N,-1.8649983297,3.3242693575,0.3668939438\H,-0.9043355222
,3.0644408396,0.5569644342\S,-3.7447268141,-0.986251276,1.4804598977\O
,-2.874772302,-2.1648419927,2.2677661006\H,-2.220206008,-2.5295029562,

1.6435210267\H,-1.1339499472,4.6288211197,-1.0394882535\C,-3.580125178
8,5.9019998026,0.010481629\H,-2.7106904668,6.0828049908,0.6468198493\H
,-3.9356143218,6.8615553413,-0.3694284022\N,-4.6614888501,5.3355888852
,0.9026073411\H,-5.5854133615,5.4332944604,0.4707861255\H,-4.466097978
2,4.2658840377,1.0130414568\\Version=EM64L-G09RevC.01\State=2-A\HF=-89
4.9110188\S2=0.767459\S2-1=0.\S2A=0.75008\RMSD=6.564e-09\RMSF=4.689e-0
6\Dipole=0.1829882,3.6941324,-0.6714356\Quadrupole=-6.7055154,19.33588
94,-12.630374,-14.0653529,-1.1333077,-4.4451462\PG=C01 [X(C6H14N2O2S1)
]\\@

**Supplemental References**

**S1**. Mentinova, M.; Barefoot, N.; McLuckey, S. *J. Am. Soc. Mass Spectrom*., **23**, 282-289, (2012).
**S2**. Moss, C. L.; Chung, T. W.; Wyer, J. A.; Nielsen, S. B.; Hvelplund, P.; Tureček, F. Dipole-Guided Electron Capture Causes Abnormal Dissociations of Phosphorylated Pentapeptides. *J. Am. Soc. Mass Spectrom.* **22**, 731-751 (2011).
**S3**. Phillips, J. C.; Braun, R.; Wang, W.; Gumbart, J.; Tajkhorshid, E.; Villa, E.; Chipot, C.; Skeel, R. D.; Kale, L.; Schulten, K. Scalable molecular dynamics with NAMD. *J. Comput. Chem.* **26**, 1781−1802 (2005).
**S4**. MacKerell, A. D. Jr.; Bashford, D.; Bellott, M.; Dunbrack, R. L. Jr.; Evanseck, J. D.; Field, M. J.; Fischer, S.; Gao, J.; Guo, H.; Ha, S.; Joseph-McCarthy, D.; Kuchnir, L.; Kuczera, K.; Lau, F. T. K.; Mattos, C.; Michnick, S.; Ngo, T.; Nguyen, D. T.; Prodhom, B.; Reiher, W. E. III; Roux, B.; Schlenkrich, M.; Smith, J. C.; Stote, R.; Straub, J.; Watanabe, M.; Wiorkiewicz-Kuczera, J.; Yin, D.; Karplus, M. All-atom empirical potential for molecular modeling and dynamics Studies of proteins. *J. Phys. Chem. B* **102**, 3586−3616 (1998).
**S5**. Sugita, Y.; Okamoto, Y. Replica exchange molecular dynamics method for protein folding. *Chem. Phys. Lett.* **314**, 141−151 (1999).
**S6**. Stewart, J. J. P. Optimization of parameters for semiempirical methods. V. Modification of NDDO approximations and application to 70 elements. *J. Mol. Model.* **13**, 1173-1213 (2007).
**S7**. Becke, A. D. New Mixing of Hartree-Fock and Local Density-Functional Theories. *J. Chem. Phys.* **98**, 1372-1377 (1993).
**S8**. Zhao, Y., Truhlar, D. G. The M06 suite of density functionals for main group thermochemistry, thermochemical kinetics, noncovalent interactions, excited states, and transition elements: two new functionals and systematic testing of four M06-class functionals and 12 other functionals. *Theor. Chem. Acc.* **120**, 215-241 (2008).
**S9.** Møller, C., Plesset, M. S. A Note on an Approximation Treatment for Many-Electron Systems. *Phys. Rev.* **46**, 618-622 (1934).
**S10.** Francisco, J. S. *J. Chem. Phys.*, **115**, 2117-2122 (2001).
**S11.** Hunter, E. P. L.; Lias, S. G. *J. Phys. Chem. Ref. Data*, **27**, 413-656 (1998).