NEW CLICKED THIIRANE DERIVATIVES AS GELATINASES INHIBITORS: THE RELEVANCE OF THE P1' SEGMENT

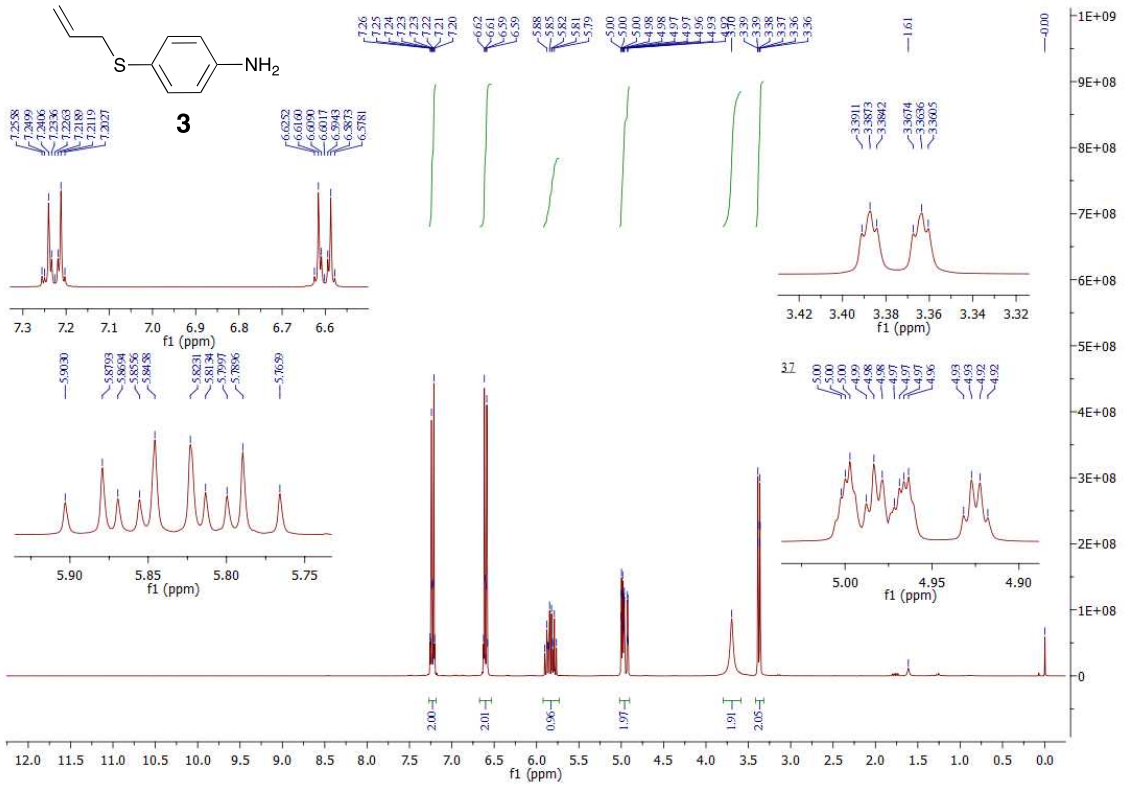
B. Fabre,[a] K. Filipiak,[a,b] C. Coderch,[a] J. M. Zapico,[a] Rodrigo J. Carbajo,[c] Anne K. Schott,[c] Antonio Pineda-Lucena,[c] B. de Pascual-Teresa,[*a] A. Ramos.[*a]

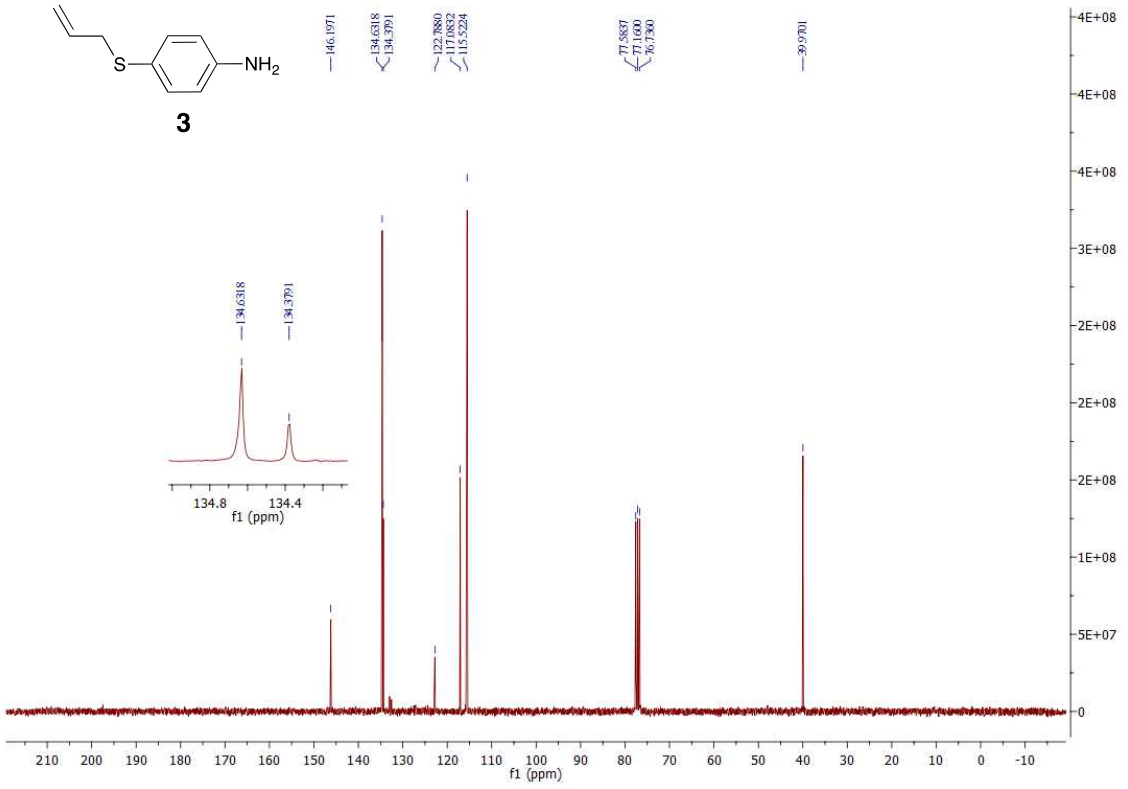
[a] Departamento de Química y Bioquímica, Facultad de Farmacia, Universidad CEU San Pablo, Urbanización Monteprincipe, 28668, Boadilla del Monte, Madrid, Spain.
[b] Department of Molecular Biology, Faculty of Biotechnology and Environment Sciences, The John Paul II Catholic University of Lublin, 20-718, Lublin, Poland.
[c] Structural Biochemistry Laboratory, Advanced Therapies Program, Centro de Investigación Príncipe Felipe, C/ Eduardo Primo Yúfera 3, 46012 Valencia, Spain

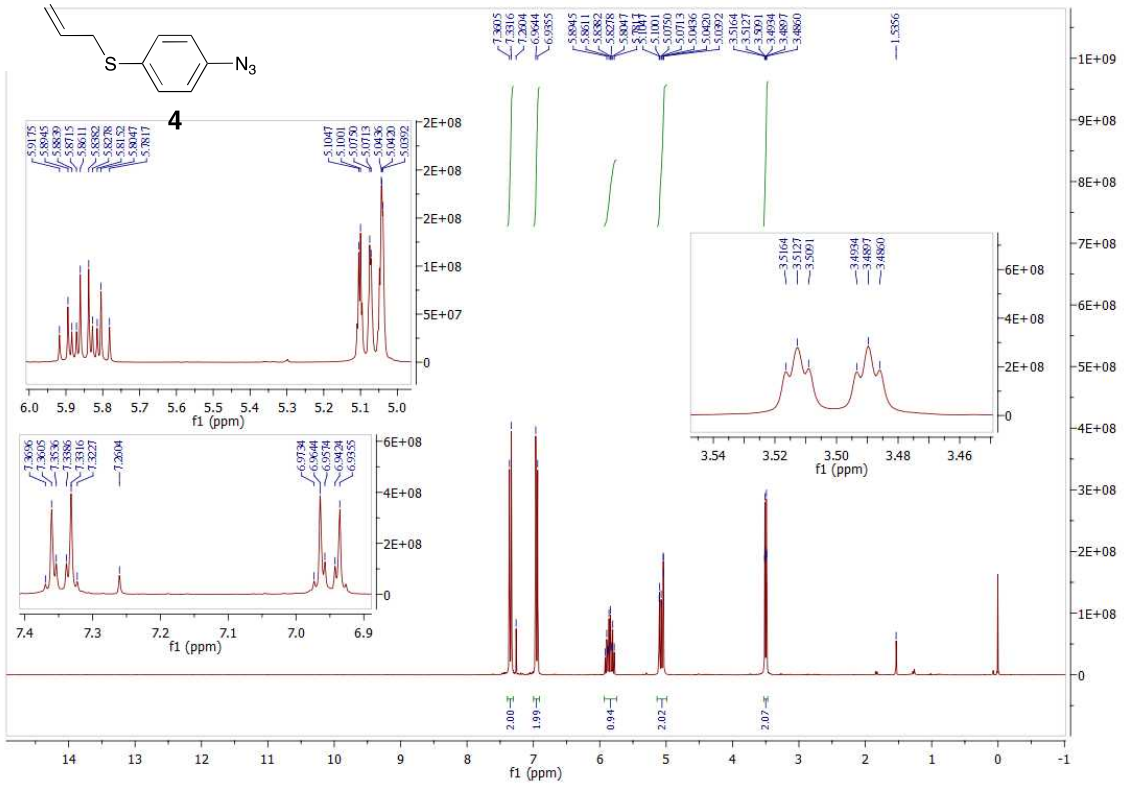
SUPPORTING INFORMATION

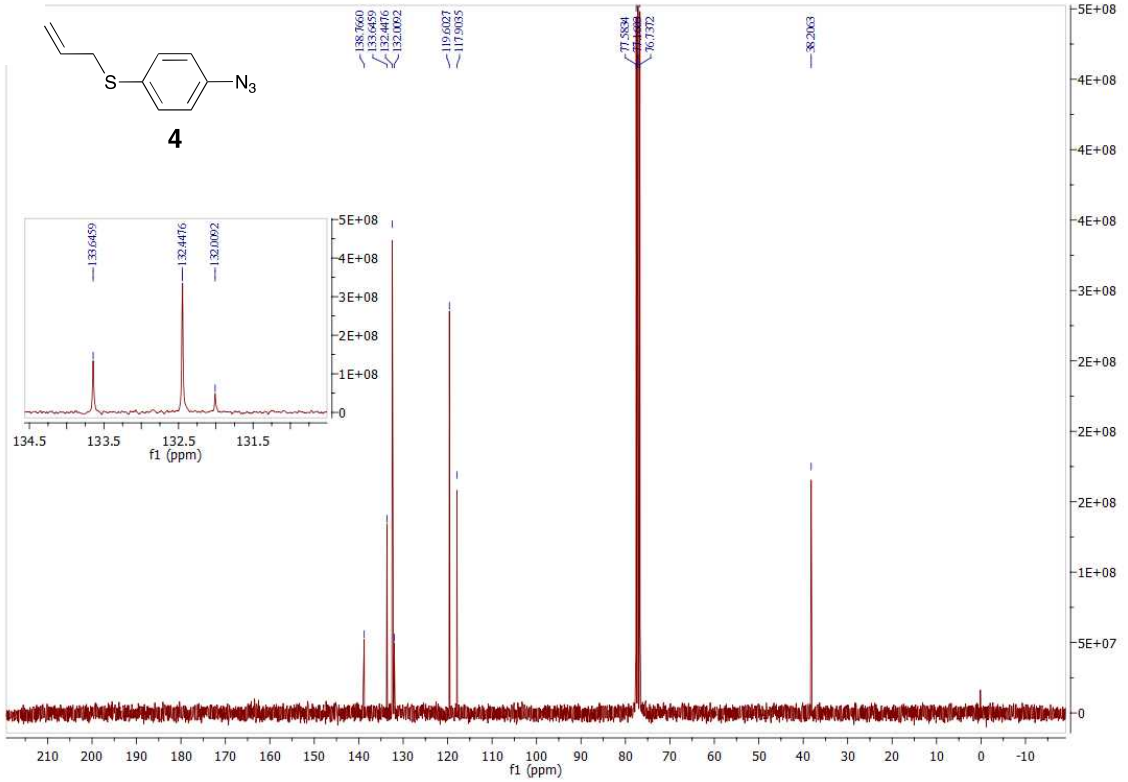
Table of Content

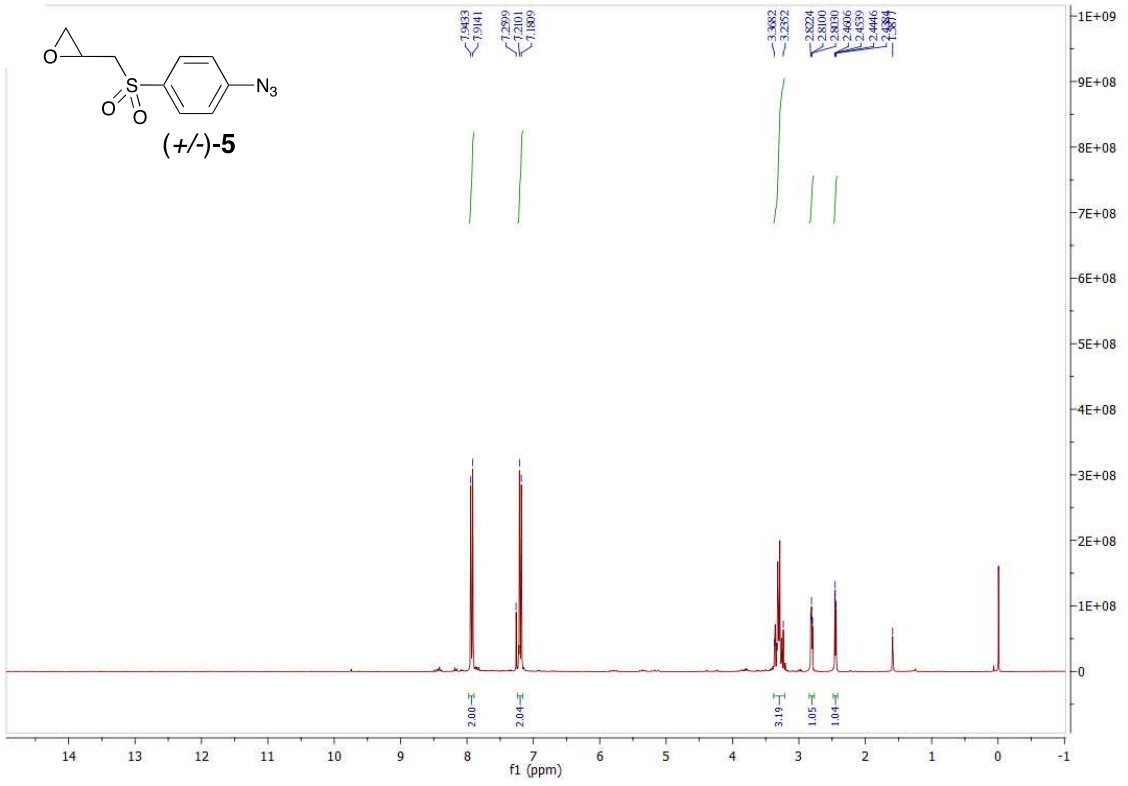
Spectral data

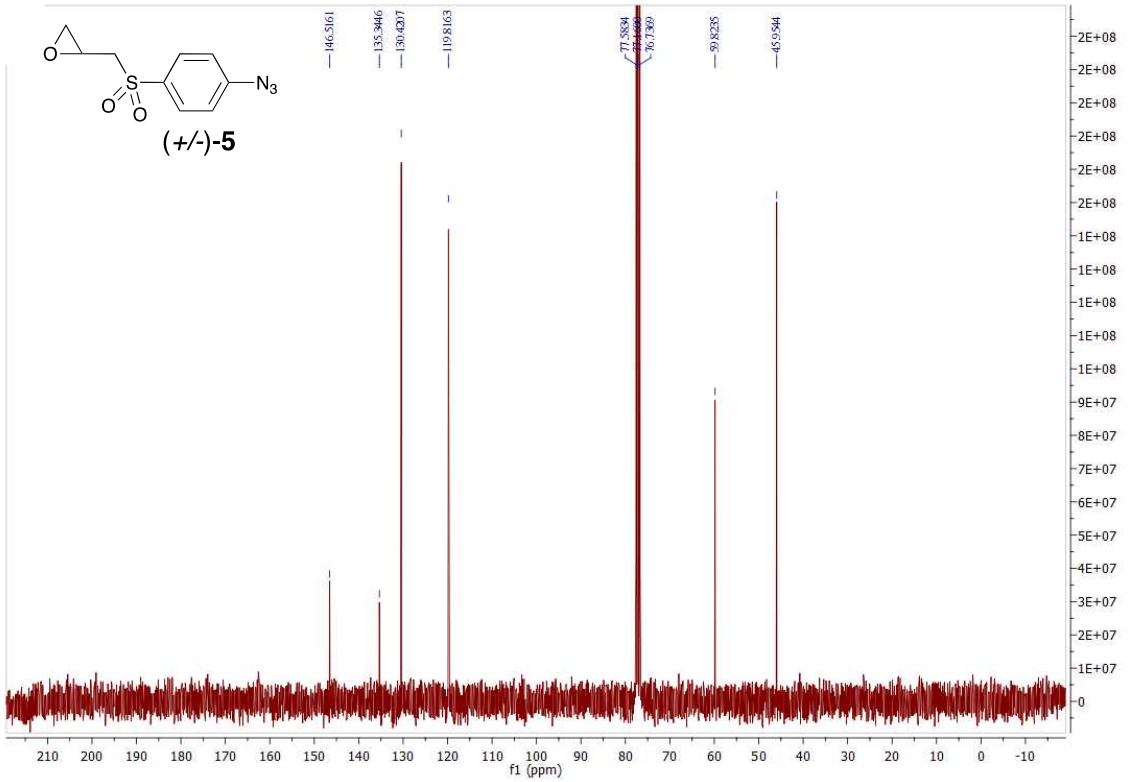


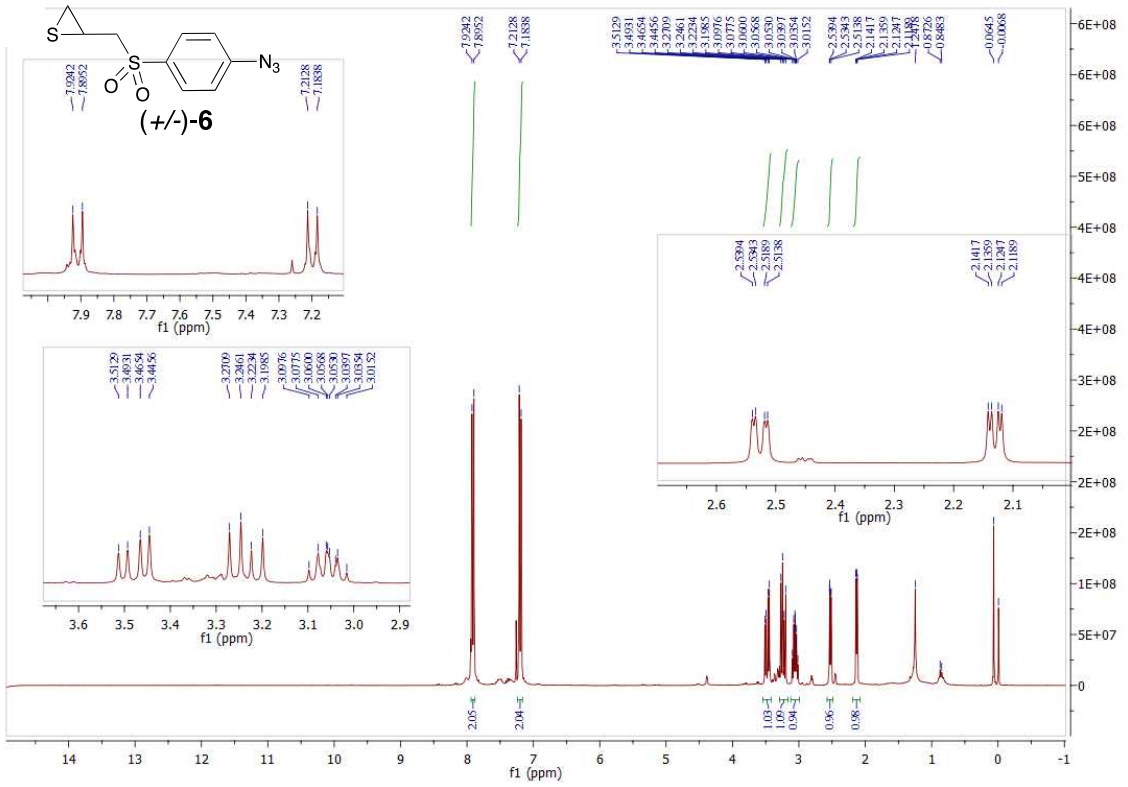


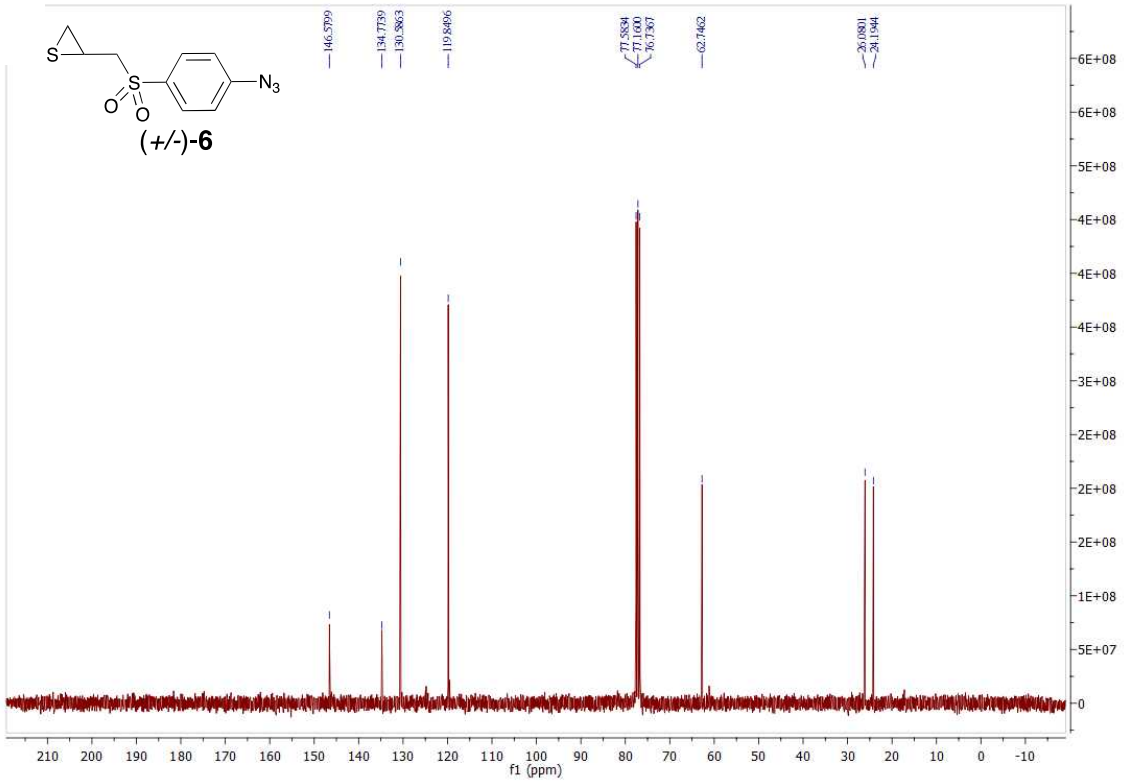

S
N3
4
f1 (ppm)

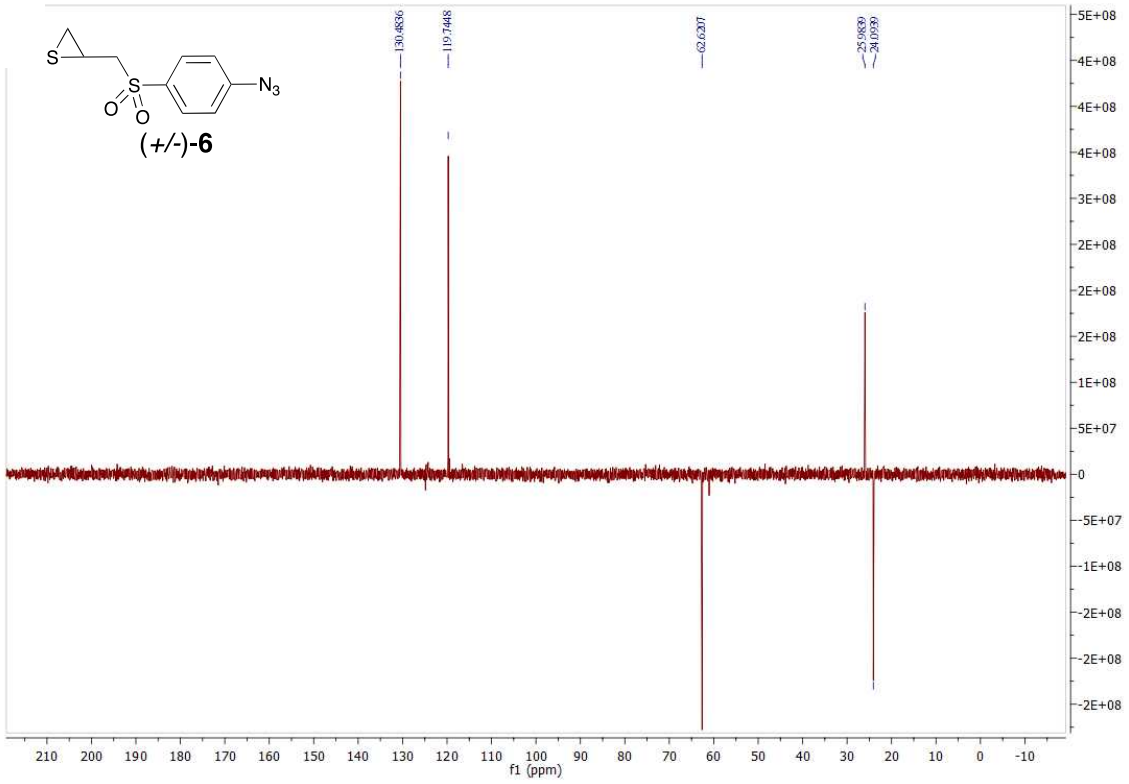

S
N3
4
f1 (ppm)

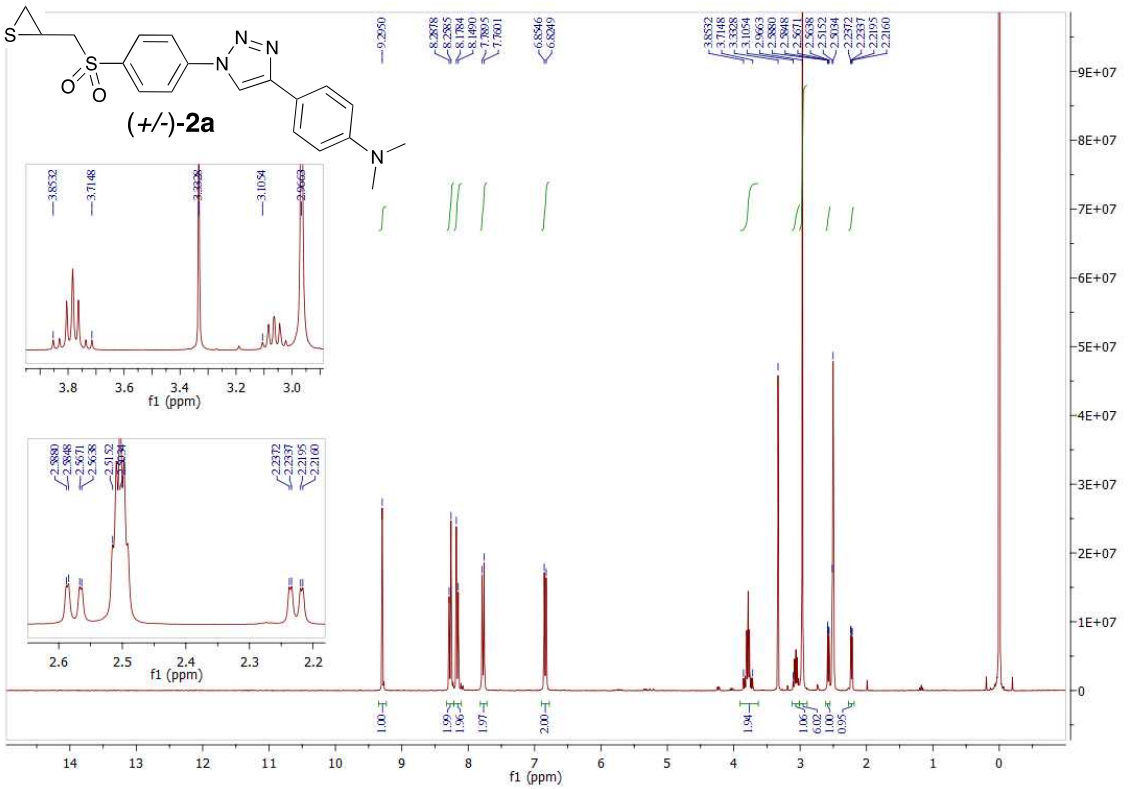

(+/-)-5
f1 (ppm)

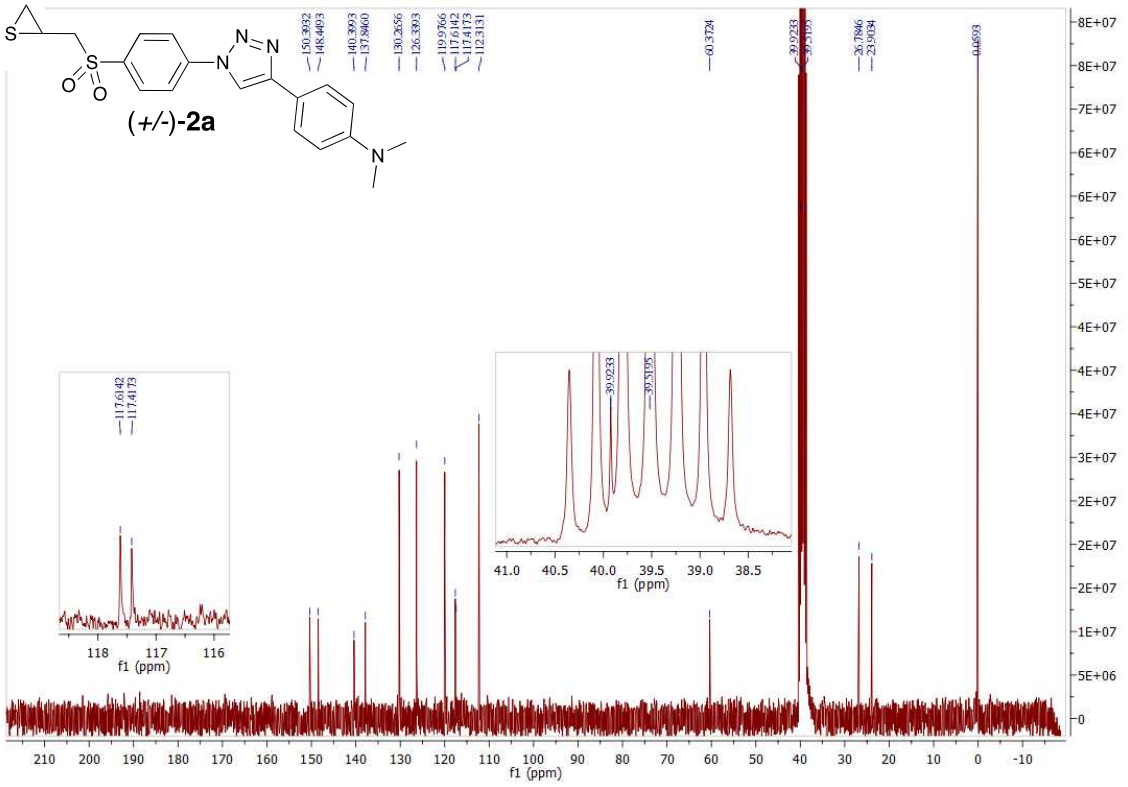

(+/-)-5
f1 (ppm)

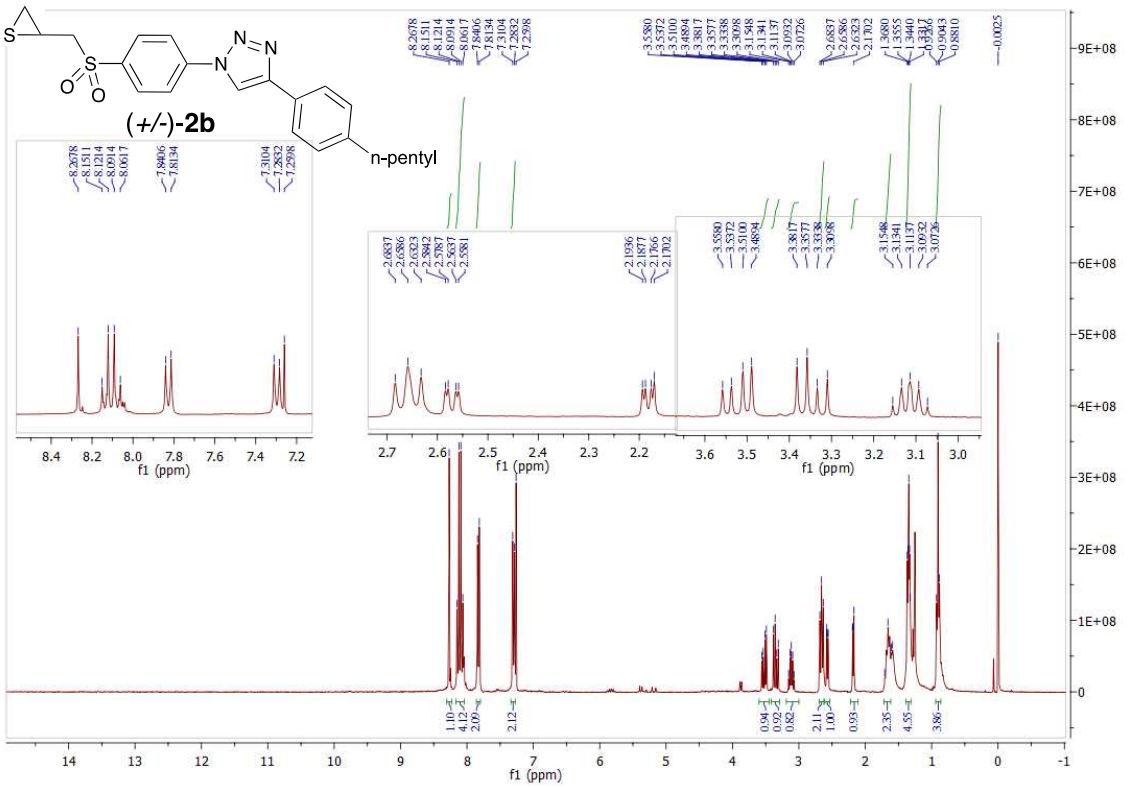

(+/-)-6
f1 (ppm)

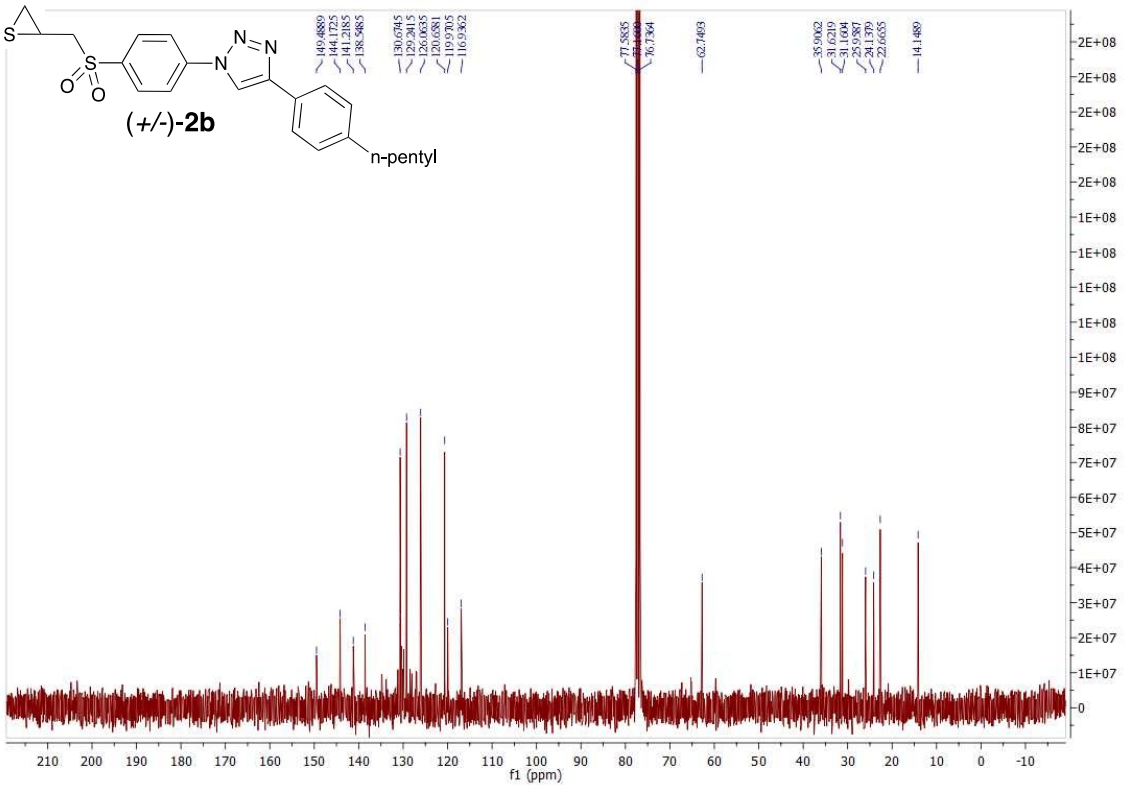

(+/-)-6
f1 (ppm)

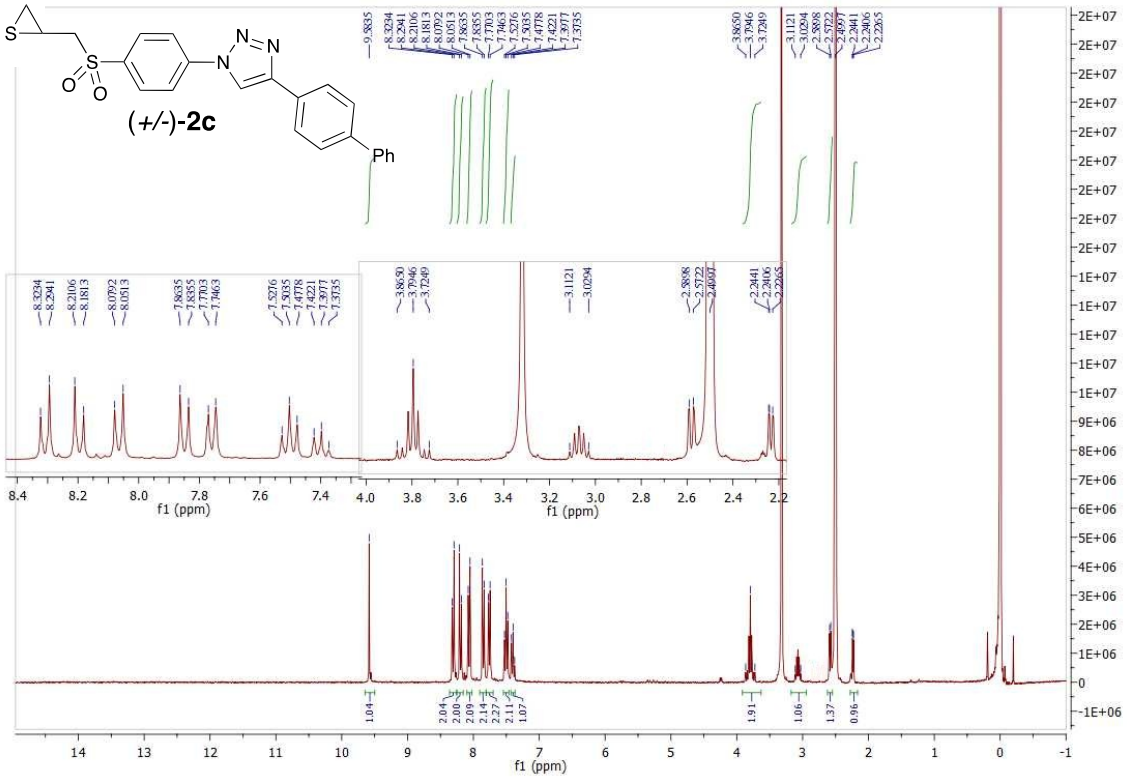

(+/-)-6
130.4836
119.7448
62.6207
25.9830
24.0939
f1 (ppm)

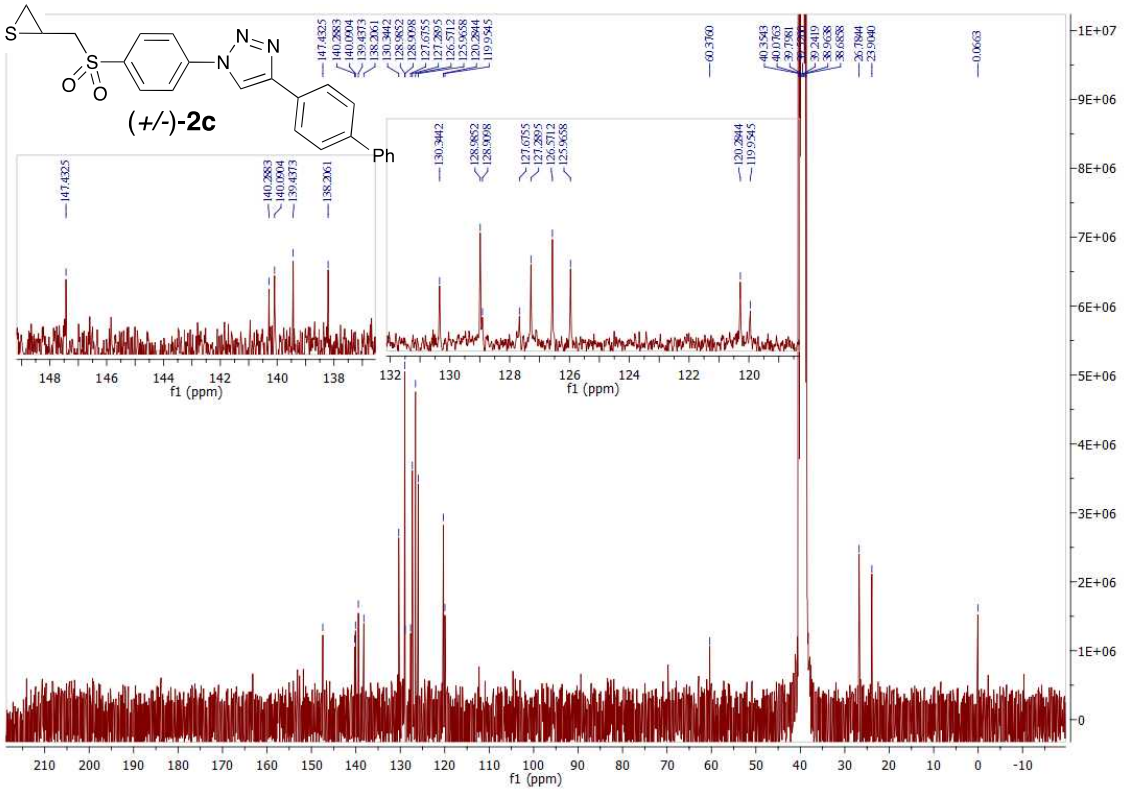

(+/-)-2a
9.2950
8.2878
8.2785
8.1784
8.1490
7.7895
7.7691
6.8546
6.8249
3.8532
3.7148
3.3328
3.1054
2.9663
2.8880
2.8488
2.8271
2.5638
2.5152
2.5094
2.2372
2.2237
2.2195
2.2160
f1 (ppm)

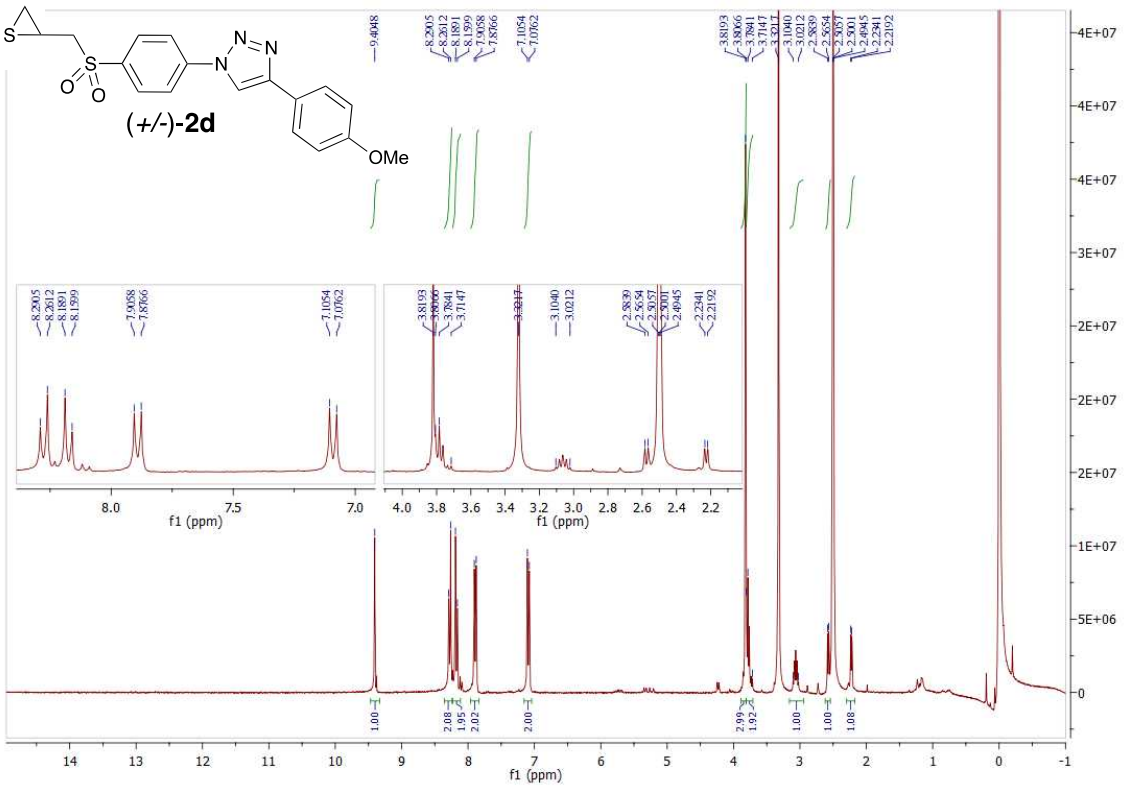

(+/-)-2a

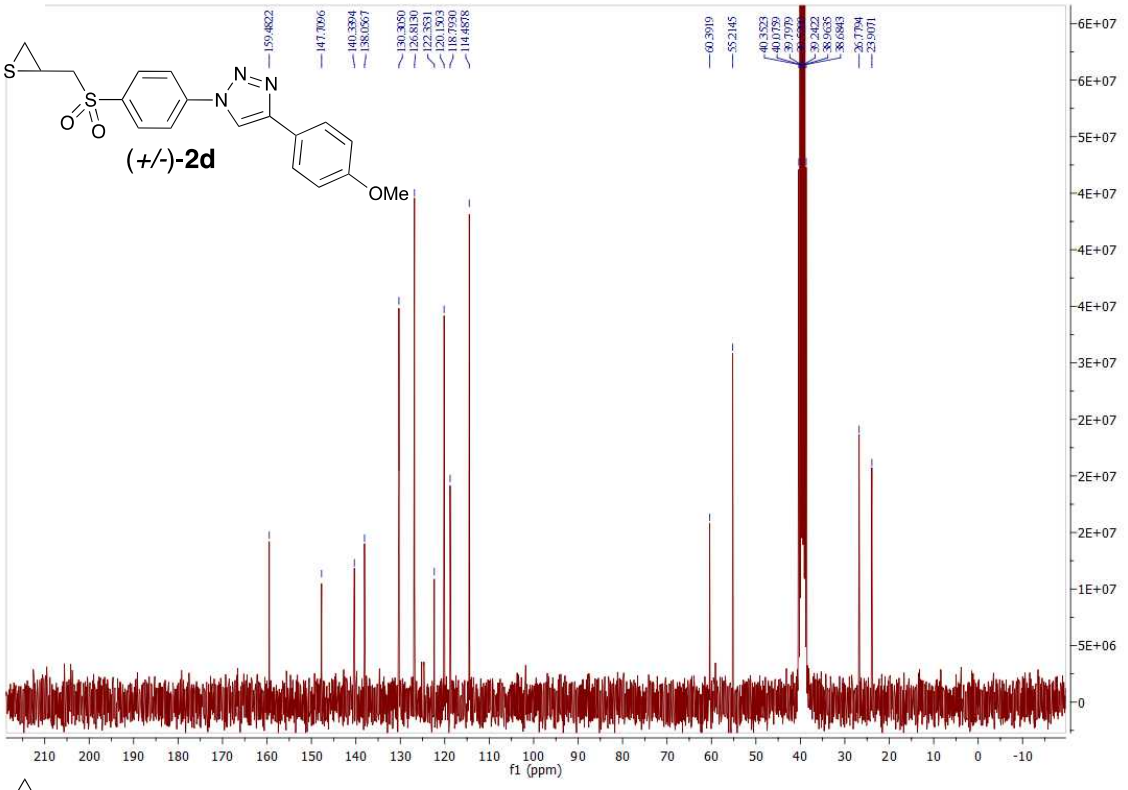

(+/-)-2b
n-pentyl

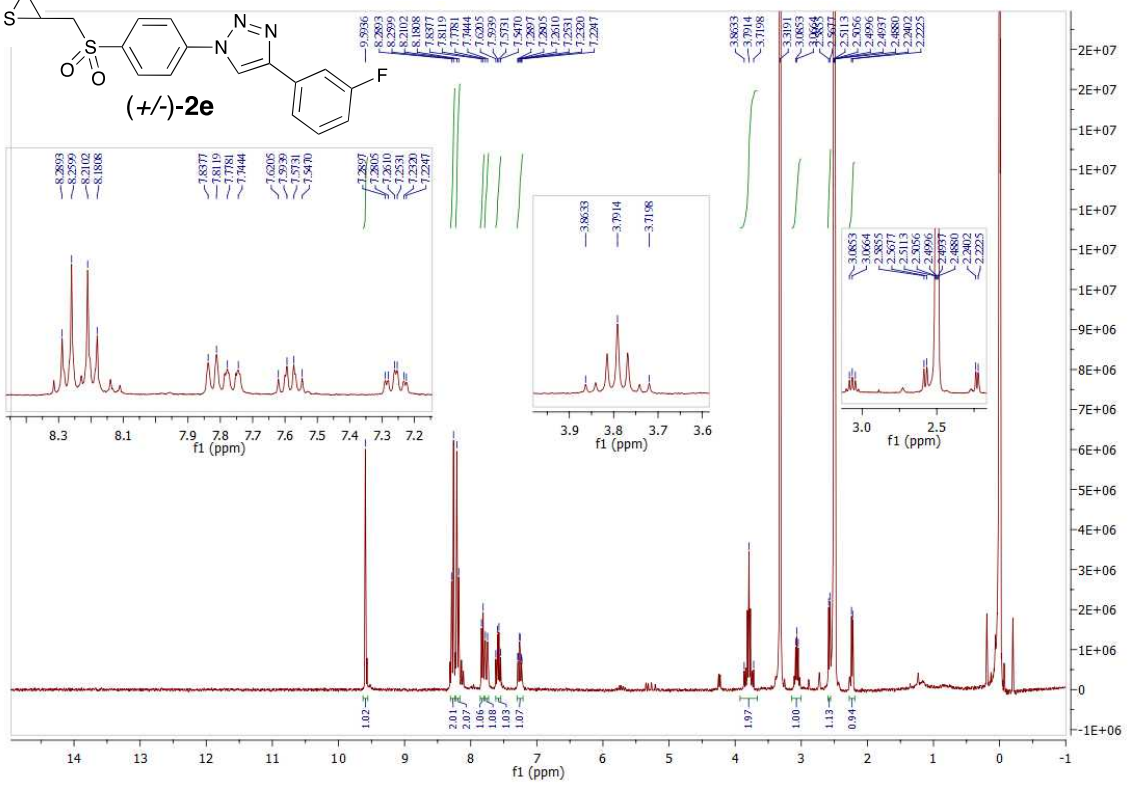

(+/-)-2b
n-pentyl
f1 (ppm)

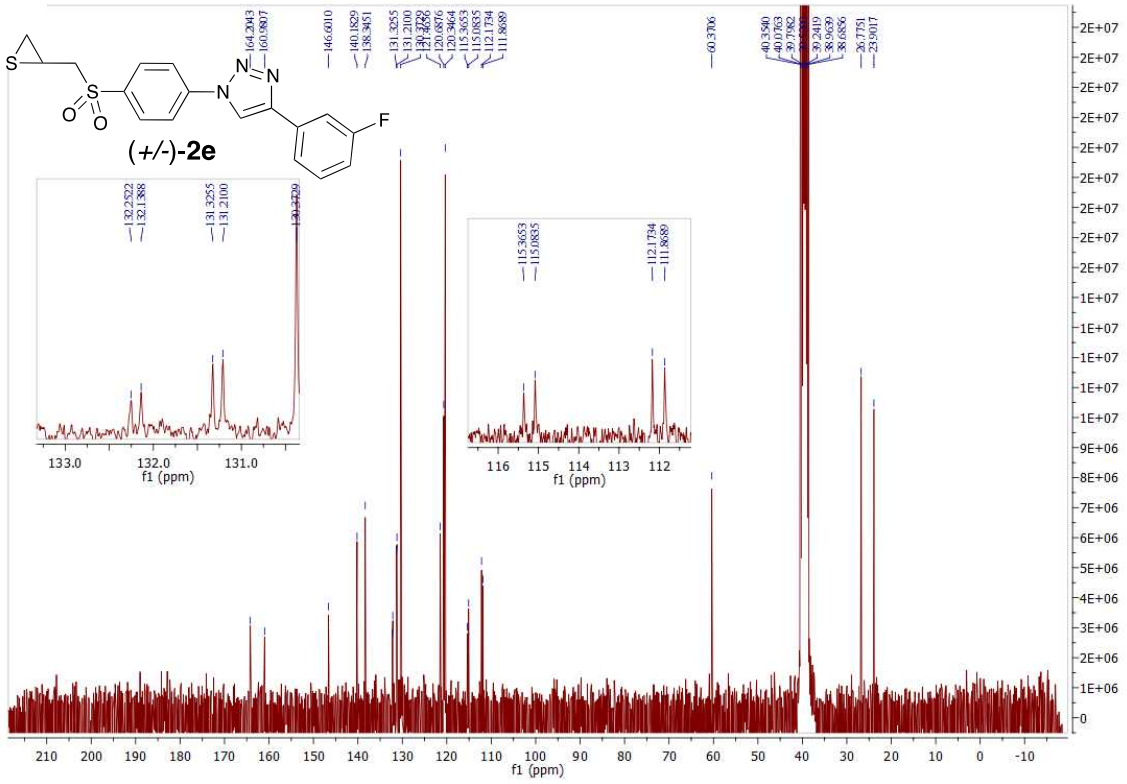

(+/-)-2c
Ph
f1 (ppm)

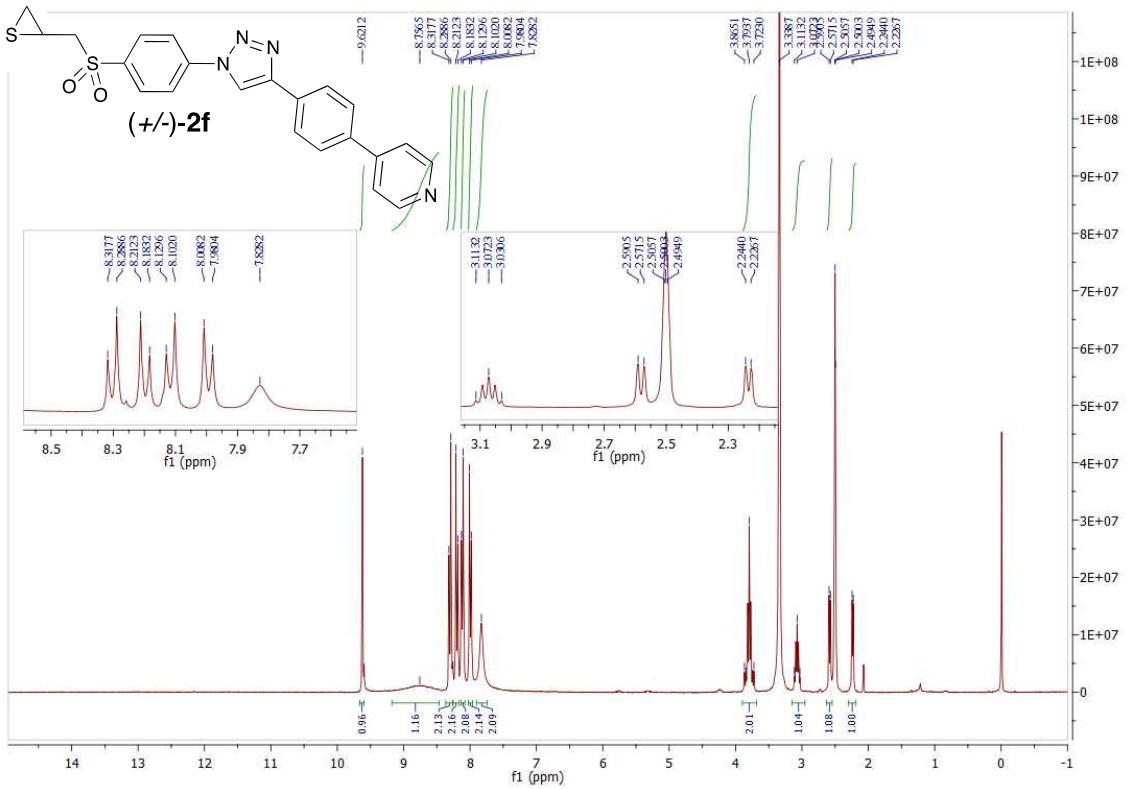

(+/-)-2c
Ph
f1 (ppm)

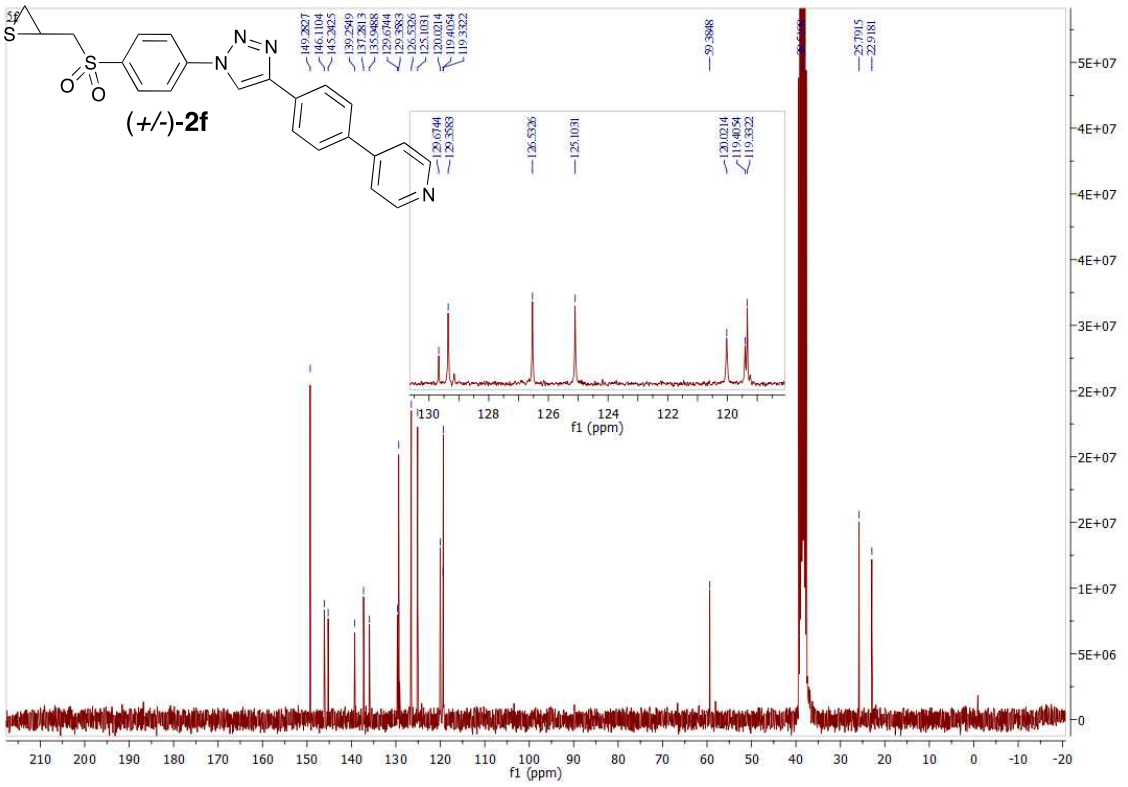

(+/-)-2d
OMe
f1 (ppm)


(+/-)-2d
OMe

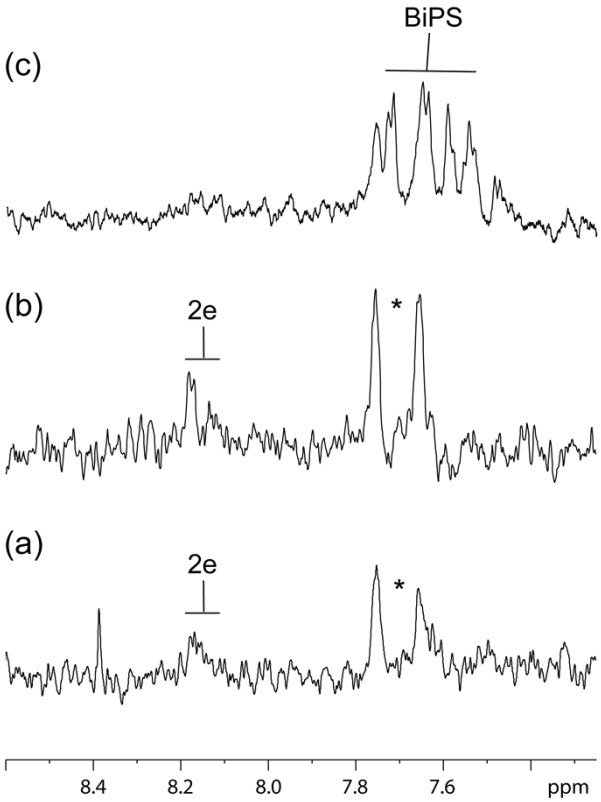

(+/-)-2e
F


(+/-)-2e
f1 (ppm)


(+/-)-2f
f1 (ppm)



MMP-2 – 2e intermolecular interaction via NMR waterLOGSY experiments



NMR binding experiments of MMP-2 with compound **2e**. (a) Monodimensional ^{1}H NMR (aromatic region) of **2e** (100 μM) and MMP-2 (5 μM). The waterLOGSY experiment of MMP-2 with **2e** (b) shows positive interaction between the compound and the protein. In the competition waterLOGSY experiment (c) the addition of the MMP-2 inhibitor BiPS (100 μM) yields a positive interaction between the protein and the inhibitor as well as the displacement of **2e** from the binding site as indicated by the disappearance of its signals from the spectrum. The resonances marked with * belong to a common contaminant from the NMR buffer.

Generation of the 1CK7 structure with relaxed S1' pocket

To obtain a structure derived from 1CK7 with a correct opening of S1' pocket, the following strategy was applied.

The docking pose of a MMP-2 hydroxamate inhibitor,[1] bearing the same P1' fragment as **2a**, in 1HOV structure[2] was superimposed to 1CK7 catalytic domain to obtain rough coordinates for this inhibitor complexed in 1CK7. The inhibitor as well as the amino acid side-chains and ions from residues beyond 10 Å of the inhibitor were relaxed, using Amber 11.[3]

To refine the catalytic zinc ion environment an additional QM/MM minimization was carried out using the version 6.0 of the Qsite software.[4] The model was solvated by a TIP3P-water cap that extended up to 20 Å from the catalytic zinc ion position. The solvent cap was relaxed by means of energy minimization and 50 ps of molecular dynamics using the sander program of the Amber11 suite[3] and the parm03 version of the all-atom AMBER force field.[5] Following the non-bonded approach, the metal ions were represented by charges (+2) and van der Waals parameters. QM-MM interfaces were placed at the Cα-Cβ link of the three conserved histidines (His_{403}, His_{408}, His_{413}) and the catalytic glutamic acid located near the catalytic zinc ion. In addition, the QM region also included the catalytic zinc ion and the inhibitor. The QM region was described at the B3LYP/LACVP* level of theory. The rest of the protein and solvent atoms were treated with the OPLS-AA force field. During QM/MM geometry optimizations, only the QM region was allowed to move while the rest of the system was kept frozen. Energy minimizations were performed without a non-bonded cutoff until the RMS gradient was less than 5.0 10^{-4} in au.

The QM/MM minimized model (without water) was surrounded by a periodic box of TIP3P water molecules that extended 15 Å from the protein atoms, and Na^+ counterions were placed to neutralize the systems. This resulted in a total of ~2500 protein atoms being solvated by ~17000 water molecules. Energy minimization and MD simulation were carried out using the SANDER and PMEMD programs included in the Amber 11 suite of programs.[3] The solvent molecules and counterions were initially relaxed by means of energy minimizations and 50 ps of MD. Then the full system was minimized to remove bad contacts in the initial geometry and heated gradually to 300 K during 60 ps of MD. The SHAKE algorithm was employed to constraint all R-H bonds, and periodic boundary conditions were applied to simulate a continuous system. A non-bonded cutoff of 10.0 Å was used and the Particle-Mesh-Ewald (PME) method was

employed to include the contributions of long-range interactions. Langevin dynamics allowed controlling the temperature (300 K) using a damping factor of 2 ps^{-1}, whereas pressure control (1 atm) used the Berendsen bath coupling. A 50 ns trajectory was computed with a time step of 2 fs and using the GPU-accelerated version of the PMEMD code included in Amber 11. The recommended SPDP precision model, which provides an optimum trade-off between accuracy and performance, was employed in the GPU-accelerated simulations. Coordinates were saved for analysis every 2 ps.

For the MD simulation, all the protein atoms were represented with the parm03 version of the AMBER force field.[5] The catalytic zinc ion, as well as the structural zinc ion and the different Ca(II) ions, were kept in the computational models. For the Ca(II) ions, we employed the non-bonded representation proposed by Aqvist.[6] For the structural zinc ion we used a set of MM parameters (bonded representation) that have been developed in the context of the parm03 force field and tested in a previous work.[7] For the catalytic zinc ion we used a mixed model. The interactions between the catalytic zinc ion and the three conserved histidines or the hydroxyl oxygen of the inhibitor hydroxamate moiety was modelled using a bonded representation in which the metal ion is linked by explicit MM bonds. The coordination of the catalytic zinc ion by the carbonyl oxygen atom of the inhibitor was represented by non-bonded parameters.

Initial and final structures of the MD simulations

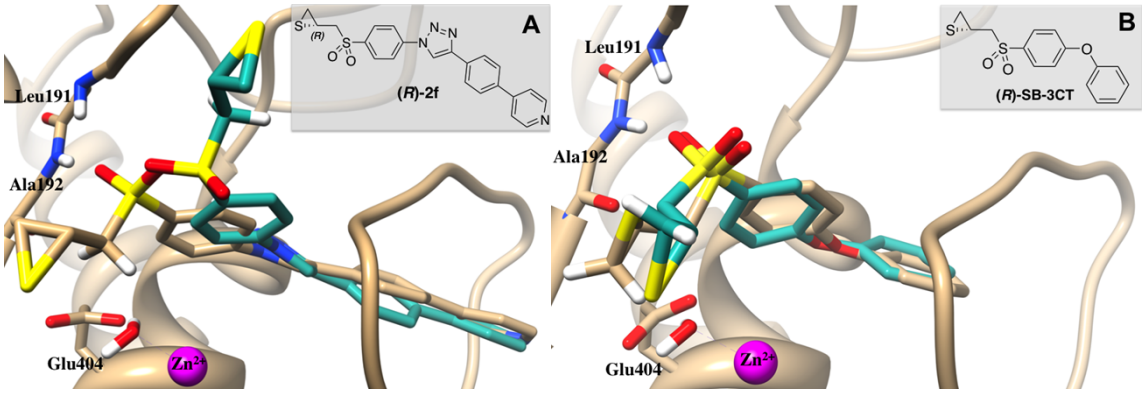


Figure S1: Superposition of the starting (tan) and final (sea green) point in our MD simulations: A) MMP-2:(*R*)-**2f** complex; B) MMP-2:(*R*)-**SB-3CT** complex. Only the backbones of the starting points are represented.

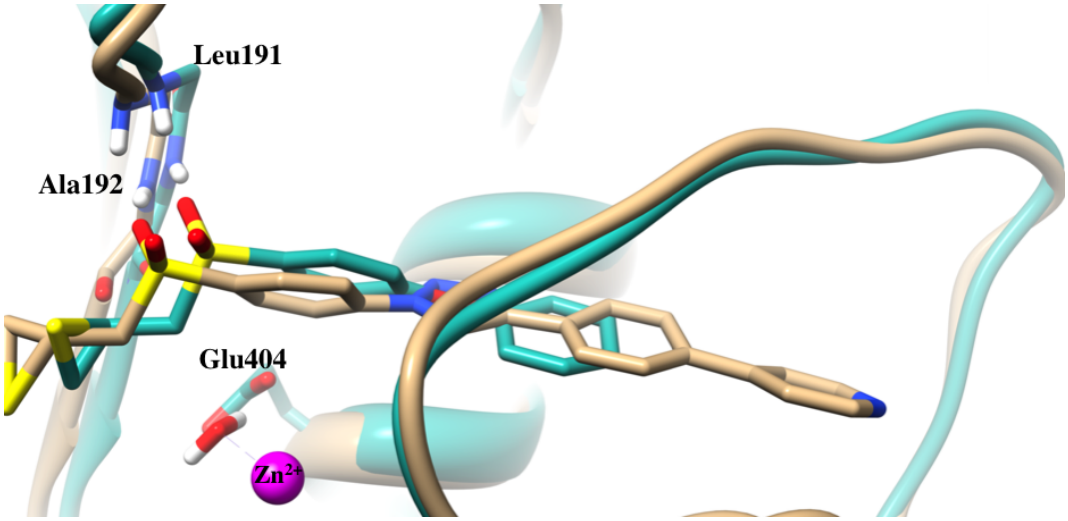


Figure S2: Superposition of the starting point of our MD simulation for the MMP-2:(*R*)-**2f** complex (tan) and the MMP-2:(*R*)-**SB-3CT** complex (sea green). Due to the longer P1' fragment, the thiirane ring of (*R*)-**2f** stands farther from the catalytic zinc ion, as well as the α-sulfone carbon from the Glu404.

References

1. B. Fabre, K. Filipiak, J. M. Zapico, N. Díaz, R. J. Carbajo, A. K. Schott, M. P. Martínez-Alcázar, D. Suárez, A. Pineda-Lucena, A. Ramos and B. de Pascual-Teresa, *Org. Biomol. Chem.*, 2013, **11**, 6623-6641.
2. Y. Feng, J. J. Likos, L. Zhu, H. Woodward, G. Munie, J. J. McDonald, A. M. Stevens, C. P. Howard, G. A. De Crescenzo and D. Welsch, *Biochim. Biophys. Acta*, 2002, **1598**, 10-23.
3. D. Case, T. Darden, T. Cheatham III, C. Simmerling, J. Wang, R. Duke, R. Luo, R. Walker, W. Zhang and K. Merz, *University of California, San Francisco*, 2010, **142**.
4. R. B. Murphy, D. M. Philipp and R. A. Friesner, *J. Comput. Chem.*, 2000, **21**, 1442-1457.
5. Y. Duan, C. Wu, S. Chowdhury, M. C. Lee, G. Xiong, W. Zhang, R. Yang, P. Cieplak, R. Luo and T. Lee, *J. Comput. Chem.*, 2003, **24**, 1999-2012.
6. J. Aqvist and O. Tapia, *Biopolymers*, 1990, **30**, 205-209.
7. N. Díaz and D. Suárez, *Biochemistry*, 2007, **46**, 8943-8952.