Conformational Analysis of the Mannosidase Inhibitor Kifunensine: A Quantum Mechanical and Structural Approach

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The varied yet family-specific conformational pathways used by individual glycoside hydrolases (GHs) offer a tantalising prospect for the design of tightly binding and specific enzyme inhibitors. A cardinal example of a GH-family-specific inhibitor, and one that finds widespread practical use, is the natural product kifunensine, which is a low-nanomolar inhibitor that is selective for GH family 47 inverting α-mannosidases. Here we show, through quantum-mechanical approaches, that kifunensine is restrained to a “ring-flipped” C1 conformation with another accessible, but higher-energy, region around the 1,2,3,4 trans conformation. The conformations of kifunensine in complex with a range of GH47 enzymes—including an atomic-level resolution (1 Å) structure of kifunensine with Caulobacter sp. CGGH47 reported herein and with GH family 38 and 92 α-mannosidasestheserevealedthatkifunensine was mapped onto the kifunensine free-energy landscape. These studies revealed that kifunensine has the ability to mimic the product state of GH47 enzymes but cannot mimic any conformational states relevant to the reaction coordinate of mannosidases from other families.

There is compelling evidence that the enzymatic hydrolysis of glycosides, catalysed by glycoside hydrolases (GHs) or glycosidases, occurs via transition states with significant oxocarbenium ion character. For pyranoside-active enzymes, Sinnott was the first to argue that the allowed canonical conformations of the transition state sugar ring were two half-chairs (H2 and H3, and their closely related envelope conformations) and two boats (B1 and B2). Sustained efforts to map the conformational reaction pathways of glycosidases leading from substrate to product via the transition state(s) have revealed that individual glycosidases are optimised to act on substrates and follow a defined conformational itinerary through a specific transition-state conformation. Glycosidases that are sequence related (for family classification see www.cazy.org and www.cazy.org) and act on sugars with the same configuration are believed to act with identical conformational reaction itineraries. It is also apparent that GHs from different families that act on substrates with the same stereochemical configuration can follow different conformational itineraries during catalysis. Given that mimicry of the enzymatic transition state is a powerful approach to inhibitor design and enzyme inhibition, the potential exists for the design or discovery of molecules with intrinsically biased conformations that could act as GH-family-specific enzyme inhibitors. However, to achieve specificity, inhibitor design does not have to be limited to transition-state mimicry but could target any distinctive conformation state along the reaction coordinate. Although highly appealing, efforts to design molecular scaffolds that predispose inhibitors to conformations matching that of the transition state of a specific GH have been disappointing. For example, molecular constraints that restrict conformational mobility typically result in steric clashes that prevent efficient binding. Moreover, a recent study that identified a unique stereoelectronic bias of manno- pyranosiduronioimidogluconononenuclides failed to deliver effective inhibition of a conformationally matched α-mannosidase, presumably because of the inability to accommodate the requisite carboxylate group. In the context of the failure thus far of rational design methods to achieve conformationally specific inhibition, one compound from nature, kifunensine, stands out as a powerful and family-specific enzyme inhibitor. Kifunensine (1; Scheme 1) is an unusual oxalamide-fused iminosugar with high specificity for α-mannosidases of GH47.

Kifunensine is produced by the actinobacterium Kitasatosporia kifunense strain no. 9482. Originally isolated on the basis of immunomodulatory properties, it was soon identified as a potent inhibitor of selected α-mannosidases. The major...
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revealed that kifunensine binds in a “ring-flipped” conformation, in line with contemporary applications of kifunensine stem from its activity as a specific inhibitor of the class I GH47 α-mannosidases involved in glycoprotein biosynthesis within the secretory pathway that is specific for the hydrolysis of α-1,2-glycosidic bonds.[9] During glycoprotein biosynthesis, glucosylated high-mannose N-glycans are appended to nascent unfolded peptide chains in the endoplasmic reticulum (ER), whereupon they undergo a range of trimming reactions in the ER that assist in folding the peptide, and subsequent trimming reactions in the Golgi apparatus that remove additional mannose residues prior to late-stage glycosylation reactions.[10] As part of this process, a quality control mechanism termed ER-associated degradation (ERAD) extracts terminally misfolded proteins from the secretory pathway for proteosomal degradation.[11] Kifunensine-sensitive α-mannosidases are found within both the normal trimming pathway (ER mannosidase I, Golgi mannosidase II), and within the ERAD pathway (ER degradation-enhancing mannosidase-like proteins 1, 2 and 3).[12]

The powerful and specific inhibition of GH47 α-mannosidases by kifunensine has led to its widespread use for manipulating the N-glycan structure. In the structural-biology context it is used to improve the homogeneity and crystallisation of proteins by arresting glycan remodelling so as to yield high-mannose glycans that are more easily cleaved by endoH.[13] Kifunensine is also used in the production of therapeutic proteins. The effectiveness of α-glucocerebrosidase as a treatment for the lysosomal storage disorder Gaucher’s disease depends upon the presence of high-mannose N-glycans of this protein, which enable ligation to mannose receptors and delivery to lysosomes.[14] The lysosomal-replacement-protein Vela-glucerase alfa (acid α-glucocerebrosidase) is produced by Shire Plc by culturing HT1080 fibrosarcoma cells expressing acid α-glucocerebrosidase in the presence of kifunensine—this promotes the biosynthesis of enzyme decorated with immature high-mannose-type N-linked glycan chains.[15]

Structural studies on GH47 enzymes, firstly the seminal work by the Howell group on the human ER ManB1 α-1,2-mannosidase,[16] and subsequent work on a Penicillium citrinum homologue[12c] revealed that kifunensine binds in a “ring-flipped” conformation. This conformation is unusual for an iminosugar. Relative to the proposed conformational pathway \((S_4 \rightarrow [H_4]^*) \rightarrow C_4\) (Scheme 2)[16,17] for this family of enzymes, it provides conformational mimicry of the product. Of relevance to this observation, a complex of noeuromycin bound to a bacterial α-mannosidase of family GH47, Caulobacter sp. K31 (CkGH47) also adopted a \(C_4\) conformation that, on the basis of computational work, was assigned as a product-mimicking species.[17a] However, in the case of noeuromycin, computational analysis of the inhibitor reveals that it favours a \(C_4\) conformation,[18] and the observed conformation on-enzyme would therefore appear to be a consequence of the enzyme restricting its shape to a higher-energy conformation. Because of the widespread application of kifunensine, and its unique specificity as a GH47-selective α-mannosidase inhibitor, we sought to understand its specificity by developing a quantitative view of the conformational preferences of this compound when isolated, relative to bound states on α-mannosidases from GH47 and other families.

Structures are available for kifunensine bound to Homo sapiens[16] and \(P. \text{citrinum}[12c]\) family 47 α-mannosidases at medium resolutions of 1.75 and 2.20 Å, respectively. In order to allow comparison to conformations that the α-mannoside substrate follows during catalysis, we sought to obtain a higher-resolution structure of 1 with CkGH47 α-mannosidase, which we have previously shown to provide atomic resolution diffraction data (Table 1). Kifunensine, shown here using isothermal titration calorimetry, is a tight binding \((K_D = 39 \text{ nM})\) inhibitor of CkGH47 (Figure 1A). Crystals of a complex of CkGH47 bound to kifunensine diffraction to 1.05 Å resolution (Figure 1B). Kifunensine binds to CkGH47 in a \(C_4\) conformation, in line with
Kifunensine is a potent inhibitor of Ck in complex with low energy zone a Ck 0.090 (0.88) [
38 (retaining), conformation. A) Thermodynamics of P. citrinum Data and structure quality for 1.

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One unusual conformational pathways: 1 conformation, with a limited ability to adopt a small O and 130 (inverting) Caulobacter 4 K H. sapiens synthesis contoured a T 1 and 1 = 1 according to methods we have Ck Golgi class II GH38 The kifunensine FEL (Figure 2A) exhibits a strong inhibitor of GH families other than family 47. es operate through es with nanomolar dissociation constants, it is usually a far poorer binder/inhibitor of GH families other than family 47. The available evidence suggests that all other exo-mannosidases operate through 0.12/0.14 125 (inverting)126 and 130 (inverting)125). Data are available for kifunensine binding to representatives of families 38 and 92. For the Drosophila melanogaster Golgi class II GH38 a-mannosid-

Table 1. Data and structure quality for 1 in complex with CKGH47 (PDB ID: 5NE5).

| Parameter               | Value       |
|-------------------------|-------------|
| Resolution [Å]          | 72–71.05    |
| Rmerge                  | 0.090 (0.88)|
| CC(1/2)                 | 1.00 (0.53) |
| Completeness [%]        | 99.2 (99.6) |
| Rwork/Rfree             | 0.12/0.14   |
| RMSD_bond, [Å]          | 0.020       |

The conformational bias of kifunensine towards the southern hemisphere is unusual for a glycosidase inhibitor. For example, FELs of the azasugar isofagomine reveal that this molecule favours a classical C4 conformation,28 which is also expected for the iminosugar deoxymannojirimycin.4 A range of imino-sugars containing sp2-hybridised atoms, best illustrated by mannoimidazole 5, favour half-chair (‘H2) and boat (B1,2) conformations that lie along the FEL tropic/equator, thus suggesting that little distortion occurs upon binding to GH47 enzymes—the apparent differences near the poles are a consequence of the deformation of the Mercator projection (for a Polar projection and direct comparison, see Figure S1 in the Supporting Information). In concordance with the FEL for 1, an FEL calculated for methyl α-mannoside within the active-centre environment of the CKGH47 (Figure 2B) revealed that this molecule is heavily distorted away from its preferred C4 conformation when bound to the enzyme.17a

Although kifunensine typically binds to GH47 α-mannosidases with nanomolar dissociation constants, it is usually a far poorer binder/inhibitor of GH families other than family 47. Although kifunensine typically binds to GH47 α-mannosidases with nanomolar dissociation constants, it is usually a far poorer binder/inhibitor of GH families other than family 47. The available evidence suggests that all other exo-mannosidases operate through 0S2−[B1,2]−−−S1 conformational pathways (GH families 2 (retaining),52 38 (retaining),22 92 (inverting),23 125 (inverting)126 and 130 (inverting)125). Data are available for kifunensine binding to representatives of families 38 and 92. The Authors. Published by Wiley-VCH Verlag GmbH & Co. KGaA, Weinheim

Figure 1. Kifunensine is a potent inhibitor of Caulobacter sp. K31 (CKGH47) α-mannosidase and binds in a 1C4 conformation. A) Thermodynamics of binding between CKGH47 and kifunensine obtained by ITC. The raw data are shown in the injection profile (top) and the titration curve (bottom); n = 0.98 ± 0.01 sites; Kn = (2.6 ± 1.1) × 102 M−1, ΔH = (19.8 ± 0.3) kcal mol−1. B) Complex with kifunensine showing the catalytic residues, the Ca2+ ion, and the nucleophilic water molecule (red sphere). The electron density map is a REFMAC maximum-likelihood/αA-weighted 2F0−F1 synthesis contoured at 0.80 electrons Å−1.
The context of the kifunensine FEL reveals that, although this conformation lies in the energetically accessible equatorial region (Figure 2A), it also lies some distance away from the $^{0}S_{2} \rightarrow [S_{2}]^{-} \rightarrow ^{1}S_{1}$ conformational pathway proposed for both GH38 and GH92 $\alpha$-mannosidases; this suggests that the poor inhibition results from an inability of the inhibitor to adopt a conformation that matches species formed on the enzyme during catalysis.

Our conformational analysis of kifunensine 1 highlights the unique ability of this compound to target a region of the FEL that is not accessed by other GH inhibitors. We suggest that the specificity and potency of 1 for GH47 family enzymes is a direct consequence of its strong preference for the southern hemisphere $^{1}C_{4}$ conformation, which matches that of the product state of the $^{3}S_{2} ightarrow [H_{S}]^{+} \rightarrow ^{1}C_{4}$ conformational itinerary that this family of enzymes is believed to follow.[2b,16,17] We highlight that this analysis suggests that potency is achieved in the absence of transition state mimicry. It is surprising that this conformational restraint is achieved by introducing an oxalamide bridge without interfering with binding in the active site, as a previous attempt with a similar goal to synthetically introduce a bridge into an iminosugar failed owing to steric clashes in the active site.[3,4] The preference of kifunensine for a $^{1}C_{4}$ conformation likely arises not merely from the fusion of the bridge to the ring, but also from the sp$^{2}$ hybridisation of the endocyclic nitrogen as part of an amide. Further, we note that kifunensine is a neutral species; this shows that, unlike most aza/iminosugar glycosidase inhibitors, kifunensine achieves its potency through shape, rather than charge. For a discussion of shape versus charge mimicry for the inhibition of a GH99 $\alpha$-mannosidase see ref. [18]; for an example of a potent class of neutral GH inhibitors see ref. [27].

In conclusion, this work highlights the capacity of natural products to provide inspiration for selectively inhibiting glycosidases based on mechanistic principles. Aligned with this goal, we highlight the inspiration provided by the natural product nagstatin (6),[28] which informed the development of the concept of lateral protonation by GHs with a catalytic acid or acid/base located anti to the C1–O5 bond, and the design of the glycoimidazole-class inhibitors that are selective for antiprotonating GHs.[29] The existence of kifunensine as a GH47-selective inhibitor should inspire continuing efforts to develop selective and potent GH inhibitors based on targeting unique conformational features of their catalysis reaction coordinates. We highlight that one other mannosidase family operates through a reversed $^{1}C_{4} \rightarrow [H_{S}]^{+} \rightarrow ^{3}S_{2}$ conformational itinerary, namely GH134 $\beta$-mannanases.[10] Based on the analysis presented herein, this family is likely to be specifically targeted by substrate-mimicking kifunensine-derived oligosaccharides in contrast to the $\beta$-mannanases of GH26 and GH113, which instead operate through $^{0}S_{2} \rightarrow [S_{2}]^{-} \rightarrow ^{1}S_{1}$ conformational pathways.[20b]

**Experimental Section**

CkGH47 protein was cloned, expressed and purified as described previously.[17a] The crystallisation conditions were the same as discussed in ref. [6]. Mature crystals were soaked in kifunensine
ICP was performed by using a MicroCal ITC200 calorimeter at 25 °C with 20 injections. CkGH47 and ikfunensine 1 were transferred into matching buffer by dialysis into HEPES (25 mM, pH 7.0) containing NaCl (50 mM) and CaCl2 (2 mM). The CkGH47 concentration in the cell was 50 μM, and the ligand concentration was 500 μM. The binding affinity was calculated by using Origin (OriginLab, Northampton, MA).

The free-energy landscape of ikfunensine was obtained by density functional theory-based metadynamics[38] by using the Car–Parrinello (CP) method.[39] The molecule was enclosed in an isolated cubic box of 14.0 x 14.0 x 14.0 Å. A fictitious electron mass of 700 au and a time step of 0.12 fs ensured a proper conservation of the total energy during the simulation. The Kohn–Sham orbitals were expanded on a plane wave (PW) basis set with a kinetic energy cutoff of 700 au.

The phase space was fully explored in less than 50 ps, and the simulation was further extended up to 95 ps. The simulation was stopped when the free energy differences among wells remained constant. The error in the energy differences of the principal minima, taken as a standard deviation (SD) from the last 30 ps, is below 0.6 kcal mol⁻¹.

Conflicts of Interest

The authors declare no conflict of interest.

Keywords: ab initio calculations — carbohydrates — enzymes — hydrolases — iminosugar

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[1] M. L. Sinnott, Chem. Rev. 1990, 90, 1171–1202.
[2] a) G. Speciale, A. J. Thompson, G. J. Davies, S. J. Williams, Curr. Opin. Struct. Biol. 2014, 24, 1–13; b) G. J. Davies, A. Planas, C. Rovira, Sci. Adv. Chem. Res. 2012, 45, 308–316.
[3] V. Lombard, H. Golaonza Ramulo, E. Drula, P.M. Coutinho, B. Henrisrat, Nucleic Acids Res. 2014, 42, D490–D495.
[4] a) V. L. Schramm, ACS Chem. Biol. 2013, 8, 71–81; b) M. M. Mader, P. A. Bartlett, Chem. Rev. 1997, 97, 1281–1301.
[5] a) M. Böhm, E. Lorthiois, M. Meyyappan, A. Vasella, Helv. Chim. Acta 2003, 86, 3818–3835; b) L.E. Tailford, W. A. Offen, N. L. Smith, C. Dumin, C. Morland, J. Gratien, M. P. Heck, R. V. Stuck, Y. Bleriot, A. Vau- la, H. J. Gilbert, G. J. Davies, Nat. Chem. Biol. 2008, 4, 306–312.
[6] E. van Rijssel, A. Janssen, A. Males, G. Davies, G. van der Marel, H. S. Overkleeft, J. Cedeño, ChemBioChem 2017, 18, 1297–1304.
[7] M. Iwami, O. Nakayama, H. Terano, M. Koshaka, H. Aoki, H. Imanaka, J. Antibiot. 1987, 40, 612–622.
[8] H. Kayakiri, S. Takase, T. Shibata, M. Okamoto, H. Terano, M. Hashimoto, T. Tada, S. Koda, J. Org. Chem. 1989, 54, 4015–4016.
[9] a) S. W. Mast, K. W. Moremen, Methods Enzymol. 2006, 415, 31–46; b) T. Kuribara, M. Hirano, G. Speciale, S. J. Williams, I. Yto, K. Totani, ChemBioChem 2017, 18, 1027–1035.
[10] R. Kornfeld, S. Kornfeld, Annu. Rev. Biochem. 1985, 54, 631–664.
[11] A. A. McCracken, J. L. Brodsky, J. Cell Biol. 1996, 132, 291–298.
[12] A. D. Elbein, J. E. Toupea, Mitchell, G. P. Kaushal, J. Biol. Chem. 1990, 265, 15599–15605; b) N. Hosokawa, I. Wada, K. Hasegawa, T. Yori- huzi, L. O. Tremblay, A. Herscovics, K. Nagata, EMBO Rep. 2001, 2, 415–422; c) Y. D. Lobiansov, F. Valleé, A. Imbert, T. Yoshiha, P. Yip, A. Herscovics, P. L. Howell, J. Biol. Chem. 2002, 277, 5620–5630; d) T. Zhou, D. Aa. Faibutt, K. W. Moremen, Y. H. Zheng, J. Biol. Chem. 2015, 290, 22184–22192.
[13] C. Yu, M. Crispin, A. F. P. Sonnen, D. J. Harvey, V. T. Chang, E. J. Evans, C. N. Scanlan, D. I. Stuart, R. J. Gilbert, S. J. Davis, Acta Crystallogr. Sect. F Struct. Biol. Cryst. Commun. 2011, 67, 785–789.
[14] B. Brumsmith, P. Salinas, B. Peterson, V. Chan, I. Silman, J. L. Sussman, P. J. Savickas, G. S. Robinson, A. H. Futerman, Glycobio 2010, 20, 24–32.
[15] T. M. Cox, Am. J. Hematol. 2013, 88, 163–165.
[16] F. Vallée, K. Karaveg, A. Herscovics, K. W. Moremen, P. L. Howell, J. Biol. Chem. 2007, 285, 41287–41298.
[17] a) A. J. Thompson, J. Dabin, J. Iglesias-Fernández, A. Ardevol, Z. Dinev, S. J. Williams, O. Bande, A. Sirivardena, C. Moreland, T.-C. Hu, D. K. Smith, H. J. Gilbert, C. Rovira, G. J. Davies, Angew. Chem. Int. Ed. 2012, 51, 10997–11001; Angew. Chem. 2012, 124, 11159–11163; b) K. Karaveg, A. Sirivardena, W. Tempel, Z.-J. Liu, J. Glushko, B.-C. Wang, K. W. More- men, J. Biol. Chem. 2005, 280, 16197–16207.
[18] M. Petricevic, L. F. Sobala, P. Z. Fernandes, L. Raich, A. J. Thompson, G. Bernardo-Seisiedos, O. Millet, S. Zhu, M. Sollogob, J. Jiménez-Barbero, C. Rovira, G. J. Davies, S. J. Williams, J. Am. Chem. Soc. 2017, 139, 1089–1097.
[19] A. Laio, M. Parrinello, Proc. Natl. Acad. Sci. USA 2002, 99, 12562–12566.
[20] a) A. Tankathrakot, J. Iglesias-Fernández, R. J. Williams, S. Penthaisong, S. Biały, Z. Hakki, R. C. Robinson, M. Hrmova, C. Rovira, S. J. Williams, J. R. Ketudat Cafms, ACS Catal. 2015, 5, 6041–6051; b) R. J. Williams, J. Igles-ias-Fernández, J. Stepper, A. Jackson, A. J. Thompson, E. C. Lowe, J. M. White, H. J. Gilbert, C. Rovira, G. J. Davies, S. J. Williams, Angew. Chem. Int. Ed. 2014, 53, 1087–1091; Angew. Chem. 2014, 126, 1105–1109.
[21] H. Kayakiri, S. Takase, T. Shibata, M. Hashimoto, T. Tada, S. Koda, Chem. Pharm. Bull. 1991, 39, 1378–1381.
[22] S. Numao, D. A. Kuntz, S. G. Withers, D. R. Rose, J. Biol. Chem. 2003, 278, 48074–48083.
[23] Y. Zhu, M. D. Suits, A. J. Thompson, S. Chavan, Z. Dinev, C. Dumon, N. Smith, K. W. Moremen, Y. Xiang, A. Siriwardena, S. J. Williams, H. J. Gilbert, G. J. Davies, *Nat. Chem. Biol.* 2010, 6, 125 – 132.

[24] S. Alonso-Gil, A. Males, P. Z. Fernandes, S. J. Williams, G. J. Davies, C. Rovira, *J. Am. Chem. Soc.* 2017, 139, 1085 – 1088.

[25] F. Cuskin, A. Basl, S. Ladevèze, A. M. Day, H. J. Gilbert, G. J. Davies, G. Potocki-Véronése, E. C. Lowe, *J. Biol. Chem.* 2015, 290, 25023 – 25033.

[26] N. Shah, D. A. Kuntz, D. R. Rose, *Biochemistry* 2003, 42, 13812 – 13816.

[27] S. J. Williams, V. Notenboom, J. Wicki, D. R. Rose, S. G. Withers, *J. Am. Chem. Soc.* 2000, 122, 4229 – 4230.

[28] a) T. Aoyagi, H. Suda, K. Uotani, F. Kojima, T. Aoyama, K. Horiguchi, M. Hamada, T. Takeuchi, *J. Antibiot.* 1992, 45, 1404 – 1408; b) K. Tatsuta, S. Miura, S. Ohta, H. Gunji, *J. Antibiot.* 1995, 48, 286 – 288.

[29] T. D. Heightman, A. T. Vasella, *Angew. Chem. Int. Ed.* 1999, 38, 750 – 770; *Angew. Chem.* 1999, 111, 794 – 815.

[30] Y. Jin, M. Petricevic, A. John, L. Raich, H. Jenkins, L. Portela De Souza, F. Cuskin, H. J. Gilbert, C. Rovira, F. Goddard-Borger, S. J. Williams, G. J. Davies, *ACS Cent. Sci.* 2016, 2, 896 – 903.

[31] G. Winter, *J. Appl. Crystallogr.* 2010, 43, 186 – 190.

[32] M. D. Winn, C. C. Ballard, K. D. Cowtan, E. J. Dodson, P. Emsley, P. R. Evans, R. M. Keegan, E. B. Krissinel, A. G. W. Leslie, A. McCoy, S. J. McNicholas, G. N. Murshudov, N. S. Pannu, E. A. Potterton, H. R. Powell, R. J. Read, A. Vagin, K. S. Wilson, *Acta Crystallogr. Sect. D Biol. Crystallogr.* 2011, 67, 235 – 242.

[33] P. Emsley, B. Lohkamp, W. G. Scott, K. Cowtan, *Acta Crystallogr. Sect. D Biol. Crystallogr.* 2010, 66, 486 – 501.

[34] S. McNicholas, E. Potterton, K. S. Wilson, M. E. M. Noble, *Acta Crystallogr. Sect. D Biol. Crystallogr.* 2011, 67, 386 – 394.

[35] R. Car, M. Parrinello, *Phys. Rev. Lett.* 1985, 55, 2471 – 2474.

[36] N. Troullier, J. L. Martins, *Phys. Rev. B* 1991, 43, 1993 – 2006.

[37] J. P. Perdew, K. Burke, M. Ernzerhof, *Phys. Rev. Lett.* 1996, 77, 3865 – 3868.

[38] A. Ardevol, C. Rovira, *J. Am. Chem. Soc.* 2015, 137, 7528 – 7547.

[39] D. Cremer, J. A. Pople, *J. Am. Chem. Soc.* 1975, 97, 1354 – 1358.