Ligand Strain and Its Conformational Complexity Is a Major Factor in the Binding of Cyclic Dinucleotides to STING Protein

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Abstract: STING (stimulator of interferon genes) is a key regulator of innate immunity that has recently been recognized as a promising drug target. STING is activated by cyclic dinucleotides (CDNs) which eventually leads to expression of type I interferons and other cytokines. Factors underlying the affinity of various CDN analogues are poorly understood. Herein, we correlate structural biology, isothermal calorimetry (ITC) and computational modeling to elucidate factors contributing to binding of six CDNs—three pairs of natural (ribo) and fluorinated (2'-fluororibo) 3',3'-CDNs. X-ray structural analyses of six (STING:CDN) complexes did not offer any explanation for the different affinities of the studied ligands. ITC showed entropy/enthalpy compensation up to 25 kcal mol⁻¹ for this set of similar ligands. The higher affinities of fluorinated analogues are explained with help of computational methods by smaller loss of entropy upon binding and by smaller strain (free) energy.

Introduction

In a quest for high-affinity ligands, protein–ligand interactions are often thoroughly probed experimentally and computationally to evaluate physico-chemical factors determining their binding properties. This may provide further guidance for improving ligand affinity and ultimately its biological activity in order to increase its potential therapeutic advantage. Standard approaches to quantify protein-ligand binding, including popular docking methods, are based on optimization of interactions in the protein cavity, filling „voids“ in the binding site by attaching various functional groups to ligands, counting hydrogen bonds etc. Numerous studies including our own efforts pointed out that the ligand strain/deformation energies (the energy price for adapting the bound conformation), water thermodynamics might be of the same or even larger magnitude than the enthalpic gains provided by favourable intermolecular interactions. Herein, we set out to investigate these subtleties in ligand binding on the example of one of the prime targets of current medicinal chemistry: the STING (stimulator of interferon genes) protein.

STING is a member of the cGAS-STING pathway and a receptor of cyclic dinucleotides (CDNs) that act as second messengers. It transduces signal from the cytosolic dsDNA sensor cGAS (cyclic GMP-AMP synthase) to the transcription factor IRF3 (interferon regulatory factor 3) through the kinase TBK1 (TANK-binding kinase 1) and to the transcription factor NF-κB (nuclear factor kappa-light-chain-enhancer of activated B-cells) through the kinase IKK (1 kappa B kinase). Stimulation of STING with CDNs eventually leads to induction of expression of type I interferons (INF) and other cytokines as TNFα or IL-6 which promote innate immune defenses against invading pathogens. Furthermore, cGAS-STING signaling pathway also plays a critical role in the inductions of spontaneous antitumor immunity and in a growing number of different autoimmune diseases, such as systemic lupus erythematosus (SLE), Aicardi-Goutières syndrome (AGS), or STING-associated vasculopathy with onset in infancy (SAVI).

Apart from the vertebrate CDN 2',3'-cGAMP, which is synthesized by cGAS, STING can also be activated by CDNs of bacterial origin, such as cyclic di-AMP (3',3'-c-di-AMP), cyclic di-GMP (3',3'-c-di-GMP) or 3',5'-cGAMP. These CDNs function as bacterial second messengers controlling biological processes such as biofilm development, motility, cell cycle and pathogenicity in bacteria. Furthermore, a plethora of non-natural CDNs with improved drug-like properties were synthesized; more or less complete list of the compounds can be found in the recent review.

Structurally, STING is a dimeric protein localized in the membrane of the endoplasmic reticulum (ER). The mono-
mers, consisting of 379 amino acid residues, are composed of an N-terminal transmembrane domain with four transmembrane helices (1–154), a dimerization CDNs binding domain (155–342) and a C-terminal tail (343–379).\textsuperscript{[30]} Importantly, upon activation, STING changes its conformation from open to closed and a so called lid is formed above the cyclic dinucleotide binding site (Figure 1).\textsuperscript{[22]} This conformational change is transduced over the ER membrane and only in the closed conformation can STING recruit and activate protein kinases TBK1 and IKK.

In this study, we present structural, biochemical and computational characterization of human wild-type STING in complex with natural CDNs: 3',3'-cGAMP, 3',3'-c-di-GMP and 3',3'-c-di-AMP and their fluorinated analogues at both 2' positions of the ribose ring, where the fluorine atom replaces the hydroxyl group (Figure 2). The fluorinated analogues were shown to be at least one order of magnitude more potent in inducing interferon type I expression than the corresponding non-fluorinated compounds.\textsuperscript{[31,34]}

First, we show that fluorinated ligands exhibit a significantly higher stabilization effect on STING in the thermal stability assay than the non-fluorinated ligands. This observation has been independently confirmed by measuring $K_D$ values pertinent to the STING-CDN interaction, employing the isothermal titration calorimetry (ITC). To explain the effect of fluorine substitution, we compared crystal structures of STING with all six above-mentioned ligands (i.e., we solved five CDN-STING crystal structures, whereas the sixth—STING in complex with the 3',3'-c-di-GMP—was solved by us previously, PDB entry 6S86, ref. [38]). Surprisingly, all the ligands were positioned in the binding pocket in (almost) exactly the same way; also, the protein seemed to adopt the identical conformation. As such, it has not provided any plausible explanation for the profound difference between potency of fluorinated and non-fluorinated ligands. Quite surprising data, though not entirely unprecedented in the literature,\textsuperscript{[35]} were obtained by ITC. Entropy/enthalpy compensation observed amounted up to 25 kcal mol$^{-1}$. Computational analyses (QM/MM modeling, DFT-D3/COSMO-RS calculations of ligand strain energies and ligand entropy calculations) provided a plausible rationale for the observed thermodynamics of binding. In summary, we explain in detail the complexity of the binding process and interplay of various

![Figure 1. C-terminal CDN binding domain of human wild-type STING in open and closed conformations. A) Unliganded STING in an open conformation (PDB entry 4EMU)\textsuperscript{[21]} B) STING in complex with its natural agonist 2',3'-cGAMP in a closed conformation (PDB entry 4KSY).\textsuperscript{[20]} Displayed distances are measured between Cα atoms of Gln184 of each monomer.](image)

physico-chemical contributions to the overall binding free energy ($\Delta G_{\text{bind}}$). At the same time, we show that $\Delta G_{\text{bind}}$ is amenable to quantitative analysis by combining structural biology, thermodynamic measurements and advanced computer modeling. We also argue that use of quantum mechanics is imperative in quantitative characterization of ligand-binding in biomolecules, as has been demonstrated before.\textsuperscript{[7,8,36]}

This is especially true in cases such as CDNs—charged macrocycles remain a formidable challenge for force-fields, accuracy of which underlie success of methods such as MM/PBSA or MM/FEP.

**Results and Discussion**

Fluorine substitution at the ribose 2'-position improves thermal stability of CDNs-STING complexes. Differential scanning fluorimetry (DSF) was employed to investigate the thermal stability of STING in complex with 3',3'-cGAMP (cGAMP), 3',3'-c-di-GMP (cdi-GMP), 3',3'-c-di-AMP (cdi-AMP) and their 2' fluorinated analogues (further denoted as F2cXMP, X = di-A, di-G, GA). It has been shown that the difference between melting temperatures ($\Delta T_m$) values) of the
unliganded STING and STING in complex with ligand strongly correlate with binding affinities of ligands towards the STING protein and can be used as reliable estimates of binding free energy.[37] All three difluorinated analogues—F_c-di-GMP, F_c-di-AMP, and F_c-di-GAMP—thermally stabilize the STING-ligand complex to a greater degree than their unsubstituted counterparts (Figure 2). In comparison with the unliganded STING whose melting temperature \( T_m \) is 44.0 ± 0.3°C, the \( \Delta T_m \) is 3.1°C for STING:c-di-GAMP complex, 2.2°C for the STING:c-di-GMP and 1.1°C for the STING:c-di-AMP complexes. However, all three fluorinated analogues stabilize STING dramatically more: \( \Delta T_m \) is 9.3°C for F_c-di-GAMP, 4.6°C for F_c-di-GMP and 12°C for F_c-di-AMP.

Crystal structures of human STING in complex with 3',3'-cGAMP, 3',3'-c-di-GMP, 3',3'-c-di-AMP and their difluorinated analogues. Being intrigued by the observed increase of the thermal stability (\( \Delta T_m \)) of ligand:protein complexes of difluorinated CDNs, we solved the crystal structures of all but one of the six studied STING:CDN complexes, (one 3',3'-c-di-GMP-STING complexed that was solved and published previously, ref.[38]). In analogy with previous crystallographic studies on the STING protein, we were able to obtain crystals for the studied CDNs complexed with the STING:CDN binding domain (residues 140–343). The crystals belonged to the tetragonal space groups P4_1,2_2_2 and P4_1,2_2_2 and diffracted to 2.2–2.9 Å resolution. The structures were solved by molecular replacement using STING in complex with 2',3'-c-GAMP (PDB entry 4KSY, ref.[32]) as a search model. The structures were refined by standard procedures to good R factors and geometry, as summarized in Table S1†. We were able to trace the whole protein chain except for approximately 13 amino acids at N-terminus, a disordered loop between Pro317 and Phe323 and a disordered loop between Glu184 and Ala193. Overall, our structures are in a good agreement with the previously published structures of the STING with CDNs,[32,37,39–41] They reveal the STING-CDN binding domain in the closed conformation where the lid is formed above the ligand binding site and the distance between the two main alpha helices is shorter by approximately 10 Å in comparison to the unliganded STING (Figures 3 and S1†, S2† and S3†). To reveal conformational changes of STING when bound to common 3',3' CDN and their fluorinated analogues we aligned the corresponding crystal structures using also the structure of wild-type STING in complex with cdi-GMP (PDB entry 6S86).[38] Surprisingly, we did not observe any significant conformational changes among the six structures, neither for ligands nor for the protein (Figure 3). Correspondingly, the RMSD values between each pair of structures are very low, ranging between 0.32 to 0.64 Å.

\[ \text{IfST} = \text{Isothermal titration calorimetry. To understand differences in binding of natural 3',3'-CDNs and their difluorinated analogues, we performed thermodynamic characterization of STING-ligand complex formation using isothermal titration calorimetry (ITC). The experiments were carried out in three buffers (Tris, HEPES, PIPES) to account for a proton exchange between buffer, protein and ligand.[82] The three buffers used cover the whole spectrum of deprotonation free energies.[43,44] For cdi-AMP or F_c-di-AMP we were not able to observe any significant change of enthalpy which has been already reported before.[33] Final results, to a great extent independent of (de)protonation events upon ligand binding, are summarized in Table 1. Representative measurements are depicted in Figure 4. In full accordance with the DSF values presented above, the observed \( K_D \) values are distinctly lower for fluorinated CDNs, up to two orders of magnitude for cGAMP/F_c-di-GAMP. Of a much greater surprise are individual \( \Delta H_{\text{bind}} \) and \( \Delta S_{\text{bind}} \) terms. For very similar ligands which bind to STING in an almost identical manner (c.f. X-ray structures discussed above), the observed entropy/enthalpy

| Ligand | cGAMP | F_c cGAMP | c-di-GMP | F_c-di-GMP |
|--------|-------|-----------|----------|-----------|
| \( K_D \) [M^-1] | \( (3.32 \pm 0.94) \times 10^5 \) | \( (3.99 \pm 1.73) \times 10^7 \) | \( (4.06 \pm 0.90) \times 10^5 \) | \( (2.74 \pm 0.37) \times 10^6 \) |
| \( K_D \) [nM] | 1900 ± 400 | 25 ± 14 | 2500 ± 600 | 370 ± 50 |
| \( \Delta G \) [kcal/mol] | -7.8 ± 0.1 | -10.4 ± 0.3 | -7.7 ± 0.1 | -8.8 ± 0.1 |
| \( \Delta H_{\text{bind}} \) [kcal/mol] | -2.8 ± 0.1 | 6.0 ± 0.1 | -20.4 ± 0.4 | -15.7 ± 0.1 |
| \( -\Delta S_{\text{bind}} \) [kcal/mol] | -5.0 ± 0.2 | -16.4 ± 0.4 | 12.7 ± 0.6 | 6.7 ± 0.2 |
| \( \Delta T_m \) | -0.18 ± 0.01 | -0.19 ± 0.01 | \( \approx 0 \) | \( \approx 0 \) |
compensations amount up to 25 kcal mol\(^{-1}\). Considering natural vs. difluoro CDN pairs, binding of the latter is entropically 6–11 kcal mol\(^{-1}\) more favourable. This more than compensates for enthalpy contributions, which tend to favour natural (2′-hydroxy) CDNs by 5–9 kcal mol\(^{-1}\). For F\(_2\)cGAMP this even leads to endothermic process, \(\Delta H_{\text{bind}} = 6.0\) kcal mol\(^{-1}\). Interestingly, in case of cdGAMP and F\(_2\)cdGAMP, titrations in different buffers yielded similar binding enthalpies indicating no proton transfer is involved in protein:ligand complex formation. For cdGAMP and F\(_2\)cdGAMP, ITC titrations in varying buffers predicted that 0.2 protons are released from the complex upon formation.

**Computational analysis.** Our structural analysis did not reveal any significant difference between the STING protein structures bound to fluorinated (2′fluororibo) or natural (ribo) 3′,5′ CDN s that could, per se, explain the observed higher activity of the fluorinated CDNs (Figure 3). Moreover, the binding modes of all six ligands are remarkably similar (Figure 5).

In order to gain insight into the stronger binding of fluorinated analogues, we decided to model the process computationally. Throughout, we used „CCSD(T)-calibrated“ protocol that combines DLPNO-CCSD(T), MP2-F12 and DFT-D3(BP-86)\(^{[45]}\) complemented with COSMO-RS implicit solvation model\(^{[46,47]}\).

The overall binding process is encompassed by large conformational changes of the protein, making it very difficult to model directly. Instead, we used different approximations to model individual steps of the process. The thermodynamic cycle used is presented in Figure 6. In order to gain additional support for our analysis, we complemented our study with calculations of 2′-deoxy analogues (see Figure S4), which are presented in the Supporting Information material (Tables S2 and S3).

### Step 1: Ligand Conformational Entropies.

The first step of the thermodynamic cycle shown in Figure 6 restricts the conformational ensemble of an unbound ligand to a single, lowest-energy conformer. The conformational flexibility of all ligands can be inspected visually (Figure S5†). Alternatively, the number of unique clusters (and their energies) is used to estimate entropic cost [see Equations (S2) and (S3) in Computational Details] of restricting the ensemble of ligands in solvent to a single structure. We approximate the overall free energy cost of this step with this entropic term, i.e. \(-T\Delta S_{\text{conf}} \approx G_{\text{conf}}\). This cost, summarized in Table 2, is consistently lower for fluorinated ligands.
only the changes in solvent, as the conformational entropy of the ligand itself has been discussed separately in the first step. The strain enthalpies are lower for natural ligands in all three cases. At the same time, the entropic term consistently disfavours natural ligands. The 2'-hydroxyl can form both intramolecular and solute-solvent hydrogen bonds. The hydrogen bond formed with the solvent does impose restrictions on organization of solvent, resulting in higher entropic penalty of non-fluorinated ligands. On the other hand, the fluorinated analogues lack these solute-solvent interactions, resulting in comparatively lower entropic penalty. The same holds true for the 2'-deoxy analogues, see Table S2.

Step 3: Protein–ligand interactions. A cluster model of the binding site derived from an optimized QM/MM model (equivalent to QM region and comprising \( \approx 600 \) atoms) was used for evaluating interaction of ligands with the protein. Calculation of interaction energy requires a reference state for both the ligand and the protein. The reference state for ligand has been obtained in the first two steps of our thermodynamic cycle. The reference state of the protein is identical for all ligands (unliganded protein structure). Thus, we focus on \( \Delta \Delta G_{\text{int}} \), that is, the difference between the interaction energies for a pair of ligands (natural and fluorinated), in which the term for the reference state of the protein cancels out [Eq. (1)].

\[
\Delta \Delta G_{\text{int}} = \Delta G_{\text{int}}^{\text{natural}} - \Delta G_{\text{int}}^{\text{di-F}}
\]

In this convention, positive values imply preference for fluorinated ligands, while negative values imply preference for natural ligands. The values of \( \Delta \Delta G_{\text{int}} \) are summarized in Table 3. The negative values of \( \Delta \Delta G_{\text{int}} \) can be interpreted as natural ligands being favoured as a result of specific interactions of the hydroxyl group with the protein, which are absent in the case of the fluorinated ligands. Similarly, the absence of specific interactions in this region for 2'-deoxy analogues results in values of \( \Delta \Delta G_{\text{int}} \) similar to those of fluorinated ligands, see Table S3.

| Ligand | \( -\Delta S_{\text{conf}} \) | \( \Delta H_{\text{strain}} \) | \( \Delta S_{\text{strain}} \) | \( -\Delta S_{\text{strain}} \) |
|--------|-----------------|-----------------|-----------------|-----------------|
| cGAMP  | 1.0             | 9.9             | 7.4             | 2.5             |
| F_dGAMP| 0.7             | 11.1            | 12.7            | 2.1             |
| cdi-GMP| 1.1             | 9.4             | 7.1             | 2.3             |
| F_dcdi-GMP| 0.6        | 10.5            | 10.4            | 0.1             |
| cdi-AMP| 1.2             | 8.8             | 5.5             | 3.2             |
| F_dcdi-AMP| 0.7         | 6.0             | 7.1             | 1.1             |

Step 2: Ligand–Strain Free Energies. In the second step, we account for the strain that has to be applied to the ligand to change from the most probable conformation in solvent (referred to as the global minimum, see Computational Details for detailed definition) to the bound-like conformation (obtained via refining X-ray models with QM/MM energy minimization). The strain Gibbs free energies are summarized in Table 2. Despite high structural similarity of both the global minima and the bound-like conformations, the strain free energies are consistently lower for fluorinated ligands.

Next, we may dissect the strain free energies into enthalpic and entropic contributions. The partitioning is obtained from temperature dependence of free energies as modelled by the COSMO-RS solvation model. The enthalpic contribution represents the energetic cost of changing the ligand conformation as well as enthalpic change in the interaction with solvent. The entropic contribution reflects...
It is important to reiterate that this step of the cycle is modeled by a stationary cluster model. As a result, the \( \Delta G_{\text{int}} \) term is mostly enthalpic and, thus, incomplete. The entropy contributions from the protein:ligand complex are not accounted for in our model and incredibly difficult to model in general. However, we speculate that similarly to the case of the strain free energies (the second step of our thermodynamic cycle discussed above), the entropic and enthalpic components partially compensate each other. Thus, the specific interactions of the 2'-hydroxyl that favour the natural ligands enthalpically may be expected to favour the fluorinated ligands entropically.

The calculated \( \Delta G_{\text{calc}}^{\text{act}} \) values and the experimental \( \Delta G^{\text{exp}} \) and \( \Delta T^{\text{int}} \) values are summarized in Table 3. The mere summation of the contributions from the individual steps should be interpreted with caution, as it lacks the entropic part of the protein:ligand interaction. Moreover, each of the three calculated steps is addressed using different set of approximations and, hence, we do not expect the values for individual steps to scale consistently.

The analysis above shows that the difluorinated ligands are favoured by lower conformational flexibility of the free ligand as well as lower entropy cost imposed by specific interactions with the solvent and the STING. This preference is only partially countered by stronger enthalpic interactions of the parent ligands with the protein. The observed higher preference for difluorinated ligands implies that it is the entropic term that prevails. This is in full agreement with the large and non-intuitive enthalpy/entropy compensation revealed by ITC measurements.

Conclusion

A series of six ligands – 3',3'-cGAMP, 3',3'-cdi-GMP, 3',3'-cdi-AMP and their difluorinated analogues—and their binding to human STING were studied from several perspectives. The \( \Delta T^{\text{int}} \) values obtained using differential scanning fluorimetry (DSF), support previously published data presenting higher potency of 2'-fluororibo 3',3' CDNs in interferon type I induction.\(^{[10,33,34]} \) Therefore, we solved three pairs of crystal structures of STING in complex with natural and difluorinated CDNs. However, comparison of these six crystal structures did not reveal any significant structural differences that could explain the extraordinary properties of difluorinated ligands. The thermodynamic characterization of the STING:ligand complex formation by ITC provided \( K_{\text{ij}} \) values distinctly lower for difluorinated CDNs, in accordance with the DSF data. Intriguingly, the \( \Delta H \) values favour natural CDNs, suggesting a huge entropy compensation for the difluorinated ligands.

Computational analysis provided plausible rationalization of this phenomenon. The difluorinated analogues seem to be favoured entropically due to their lower conformational flexibility and organization of solvent around the molecules. This advantage is partially compensated by weaker interaction with the protein. Comparing the magnitude of conformational and strain free energies, \( \Delta G_{\text{calc}}^{\text{act}} + \Delta G_{\text{calc}}^{\text{exp}} \) (i.e., the ligand-only part of the \( \Delta G_{\text{calc}}^{\text{act}} \)) shows that the properties of the ligand itself do play a prominent role, but the resulting affinity of the ligand to this protein is a complex interplay of both the ligand and protein-ligand properties. This highlights that design of ligands can benefit heavily from thorough conformational analysis performed at high level of theory—using standard, but advanced methods of quantum chemistry.

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Conflicts of interest

The authors declare no conflict of interest.

Stichwörter: conformational analysis · cyclic dinucleotides · entropy · quantum chemistry · strain energy

References

[1] Y. B. Shan, E. T. Kim, M. P. Eastwood, R. O. Dror, M. A. Seeliger, D. E. Shaw, J. Am. Chem. Soc. 2011, 133, 9181–9183.
[2] U. Ryde, P. Soderhjelm, Chem. Rev. 2016, 116, 5520–5566.
[3] „What Next for Quantum Mechanics in Structure-Based Drug Discovery?“: R. A. Bryce in Quantum Mechanics in Drug Discovery (Ed.: A. Heifetz), Springer US, New York, 2020, pp. 339–353.
[4] A. Sandner, T. Hufner-Wulsdorf, A. Heine, T. Steinmetzer, G. Klebe, J. Med. Chem. 2019, 62, 9753–9771.
[5] Y. Wang, J. Kim, C. Hilty, Chem. Sci. 2020, 11, 5935–5943.RETURN TO ISSUEPREVARTICLENEXTRATIONAl Design of Novel Highly Potent and Selective Phosphatidylinositol 4-Kinase IIIβ (PI4KB) Inhibitors as Broad-Spectrum Antiviral Agents and Tools for Chemical Biology
[6] I. Mejídrová, D. Chalupská, P. Plačková, C. Müllner, M. Šála, M. Klima, A. Baumlová, H. Hřebaběcký, E. Procházková, M. Dujmek, D. Strunin, J. Weber, G. Lee, M. Matoušová, H. Mertliková-Kaiserová, J. Ziebuhr, G. Birkes, E. Boura, R. Nencka, J. Med. Chem. 2017, 60, 100–118.
[7] S. M. Eyrírméz, C. Kopruluoğlu, J. Rezac, P. Hobza, ChemPhysChem 2019, 20, 2759–2766.
[8] A. Pecina, J. Brynda, L. Vrazil, R. Gnanasekaran, M. Hořejší, S. M. Eyrírméz, J. Rezáč, M. Lepšík, P. Rezáčová, P. Hobza, P. Majer, V. Veverka, J. Fantrík, ChemPhysChem 2018, 19, 873–879.
[9] G. C. Wu, T. Zhao, D. W. Kang, J. Zhang, Y. N. Song, V. Namasivayam, J. Kongsted, C. Panneconque, E. De Clercq, V. Poongavanan, X. Y. Liu, P. Zhan, J. Med. Chem. 2015, 59, 9375–9414.
[10] L. Wang, Y. Wu, Y. Deng, B. Kim, L. Pierce, C. Krilov, D. Lupyan, S. Robinson, M. K. Dahlgren, J. Greenwood, D. L. Romero, C. Masse, J. L. Knight, T. Steinbrecher, T. Beuming, D. Damm, E. Harder, W. Sherman, M. Brewer, R. Wester, M. Murcko, L. Frye, R. Farid, T. Lin, D. L. Mobley, W. L. Jorgensen,
[31] C. Shu, G. H. Yi, T. Watts, C. C. Kao, P. W. Li, Nat. Struct. Mol. Biol. 2012, 19, 722–724.
[32] X. Zhang, H. P. Shi, J. X. Wu, X. W. Zhang, L. J. Sun, C. Chen, Z. J. Liou, Mol. Cell 2013, 51, 226–235.
[33] T. Lioux, M.-A. Mauny, A. Lamoureaux, N. Bascoul, M. Hays, F. Vernejoul, A.-S. Baudru, C. Boulara, J. Lopes-Vicente, G. Oushair, G. Tiraby, J. Med. Chem. 2016, 59, 10253–10267.
[34] „Fluorinated Cyclic Dinucleotides for Cytokine Induction”: INVIVOGEN, WO2016096174A, 2016.
[35] S. Y. Ouyang, X. Q. Song, Y. Y. Wang, H. Ru, N. Shaw, Y. Jiang, F. F. Niu, Y. P. Zhu, W. C. Qiu, K. Parvatiyar, Y. Li, R. G. Zhang, G. H. Cheng, Z. J. Liu, Immunity 2012, 36, 1073–1086.
[36] J. Antony, S. Grimmie, D. G. Liakos, F. Neese, J. Phys. Chem. A 2011, 115, 11120–11220.
[37] B. Novotná, L. Vaneková, M. Zavrel, M. Buděšínský, M. Dejmek, M. Smola, O. Gutten, Z. A. Tehrani, M. P. Polidarová, A. Brázdová, R. Liboska, I. Štěpánek, Z. Vavřina, T. Jandušík, R. Nencka, L. Rulíšek, E. Bouřa, J. Brynda, O. Páv, G. Bírků, J. Med. Chem. 2019, 62, 10676–10690.
[38] M. Smola, G. Bírků, E. Bouřa, Acta Crystallogr. Sect. F 2019, 75, 593–598.
[39] S. L. Ergun, D. Fernandez, T. M. Weiss, L. Y. Li, Cell 2019, 179, 290–301.
[40] H. P. Shi, J. X. Wu, Z. J. J. Chen, C. Chen, Proc. Natl. Acad. Sci. USA 2015, 112, 8847–8852.
[41] P. Gao, M. Ascano, T. Zillinger, W. Y. Wang, P. H. Dai, A. A. Serganov, B. L. Gaffney, S. Shuman, R. A. Jones, L. Deng, G. Hartmann, W. Barchet, T. Tuschi, D. J. Patel, Cell 2013, 154, 748–762.
[42] S. G. Krimmer, G. Klebe, J. Comput.-Aided Mol. Des. 2015, 29, 867–883.
[43] O. Bastiansen, J. J. Christensen, L. D. Hansen, R. M. Izatt, Handbook of Proton Ionization Heats and Related Thermodynamic Quantities, Wiley, New York 1976.
[44] H. Fukada, K. Takahashi, Proteins: Struct. Funct. Genet. 1998, 33, 159–166.
[45] J. Rezač, D. Bim, O. Gutten, L. Rulíšek, J. Chem. Theory Comput. 2018, 14, 1254–1266.
[46] A. Klamt, J. Phys. Chem. 1995, 99, 2224–2235.
[47] A. Klamt, V. Jonas, T. Bürger, J. C. W. Lohrenz, J. Phys. Chem. A 1998, 102, 5074–5085.

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