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Novel Poxin Stable cGAMP-Derivatives Are Remarkable STING Agonists

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Abstract: 2′,3′-cGAMP is a cyclic A- and G-containing dinucleotide second messenger, which is formed upon cellular recognition of foreign cytosolic DNA as part of the innate immune response. The molecule binds to the adaptor protein STING, which induces an immune response characterized by the production of type I interferons and cytokines. The development of STING-binding molecules with both agonistic as well as antagonistic properties is currently of tremendous interest to induce or enhance antitumor or antiviral immunity on the one hand, or to treat autoimmune diseases on the other hand. To escape the host innate immune recognition, some viruses encode poxin endonucleases that cleave 2′,3′-cGAMP. Here we report that dideoxy-2′,3′-cGAMP (**1**) and analogs thereof, which lack the secondary ribose-OH groups, form a group of poxin-stable STING agonists. Despite their reduced affinity to STING, particularly the compound constructed from two A nucleosides, dideoxy-2′,3′-cAAMP (**2**), features an unusually high antitumor response in mice.

*T*he innate immune system is the first line of defense against pathogens. It is triggered by dedicated sensor proteins that recognize specific pathogen features as non-self.[1,2] Bacterial and viral infections, but also ruptured nuclear and mitochondrial membranes of damaged cells, generate double-stranded DNA (dsDNA) in the cytosol of the corresponding cell.[3] This creates a pathogenic state that is sensed by the enzyme cyclic-GMP-AMP-synthase (cGAS), which cyclizes GTP and ATP to generate the second messenger 2′,3′-cyclic-GMP-AMP (2′,3′-cGAMP) (Figure 1a).[4–7] Binding of 2′,3′-cGAMP to the endoplasmic reticulum transmembrane protein stimulator of interferon genes (STING) leads to its oligomerization, which finally stimulates the expression of type I interferons (IFNs) and pro-inflammatory cytokines with potent anti-viral and anti-bacterial effects.[8] To circumvent the cGAS/STING host defense system, vaccinia viruses encode poxvirus immune nucleases (poxins), which were shown to specifically hydrolyze the 3′-5′-linkage of the mediator molecule 2′,3′-cGAMP, leading to its degradation (Figure 1a).[9] This is achieved by the metal ion-free catalysis of an auto-degradation process, in which the poxin activates the free 2′-OH of 2′,3′-cGAMP with an active site lysine residue (K142) to promote an intramolecular attack on the 3′-5′ phosphodiester linkage, to generate an adenosine-2′,3′-cyclophosphate intermediate. According to this mechanism, removing the 2′-OH group of 2′,3′-cGAMP would potentially provide a powerful agonist that would be resistant to this viral escape pathway. However, removing the ribose OH group has consequences regarding binding to STING. It is proposed that the 3′-OH group establishes a key interaction with Ser162 of the human STING (hSTING) active site.[10] This is supposed to allow hSTING to differentiate 2′,3′-cGAMP from 3′,3′-cGAMP, which is a key bacterial second messenger (Figure 1a).

In the context of antiviral therapies, the cGAS/STING-pathway is a key component of innate immunity against DNA viruses and retroviruses such as HIV.[11–15] Activation of STING can consequently increase antiviral responses. In

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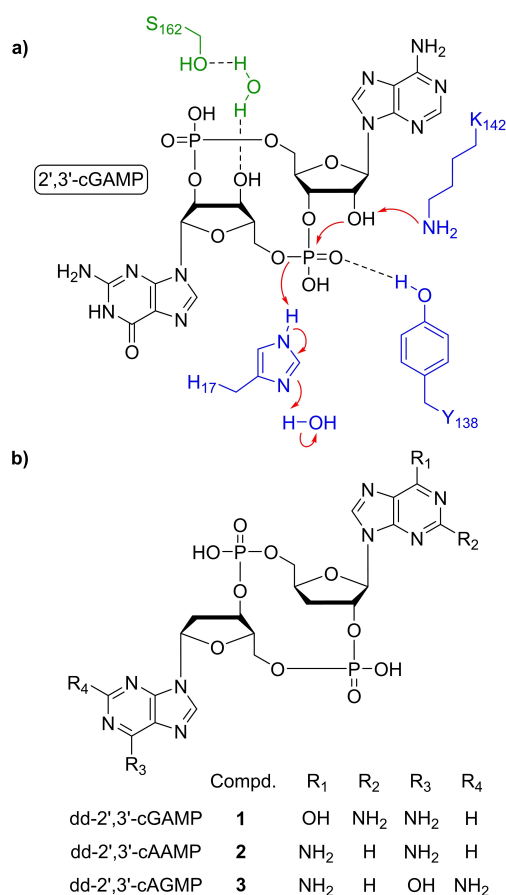[+] These authors contributed equally to this work.

Figure 1. a) Depiction of important 2′,3′-cGAMP interactions. Green: Interaction of 3′-OH with S162 of hSTING according to Zhang et al.[10] Blue: Mechanism of cleavage performed by vaccinia virus poxins, adapted from Eaglesham et al.[9] b) Structures of the synthesized dideoxy-2′,3′-cGAMP derivatives.

addition, STING activation can stimulate antitumor immune responses, which makes STING also a prime target for cancer immunotherapy.[16–19] The first STING agonists have recently entered clinical trials.[20–22] Here we present a new and concise synthesis of dideoxy-2′,3′-cyclic-dinucleotides (dd-2′,3′-CDNs) such as **1**, **2** and **3** (Figure 1b) and data about poxin-mediated degradation. Furthermore, we benchmark the synthesized compounds to natural 2′,3′-cGAMP in a preclinical mouse model of hepatocellular carcinoma.

The synthesis of dd-2′,3′-CDNs **1**–**3** started from protected xylofuranose **4** (Scheme 1). Dimethoxytrityl (DMTr) protected 3′-deoxyribonucleotides **5** and **6** were obtained over seven steps via Barton-McCombie deoxygenation, acetolysis and subsequent Vorbrüggen glycosylation, according to literature procedures.[23–25] The key 2′-phosphotriester precursors **7** and **8** were prepared in a four-step one-pot reaction by first converting **5** and **6** into the respective 2′-phosphoramidites, then condensing them with allyl alcohol, followed by oxidation with *tert*-butyl hydroperoxide (*t*BuOOH) and deprotection with dichloroacetic acid (DCA). Precursors **7** and **8** were subsequently coupled with commercially available adenosine and guanosine phosphoramidites **9** and **10** to give the 2′-5′ linked dinucleotides **11**, **12**

and **13**. Next, the allyl protecting group was removed with sodium iodide in refluxing acetone to provide the dinucleotides **14**, **15** and **16**. After precipitation and product isolation, 1-(mesitylene-2-sulfonyl)-3-nitro-1*H*-1,2,4-triazole (MSNT) was added to solutions containing **14**–**16** to activate the free phosphate for the cyclization key step, which established the 3′-5′ linkage. The raw cyclization products were not isolated, but directly subjected to a deprotection step with 33% *v/v* methylamine in ethanol. We obtained the final compounds **1**, **2** and **3** after precipitation from cold acetone and purification by reverse-phase HPLC in the form of white powders.

The binding properties of dd-2′,3′-CDNs **1**–**3** were evaluated with nano differential scanning fluorimetry (nDSF) thermal shift experiments. To this end, we added increasing amounts of the compounds to recombinant hSTING and measured the protein melting curves. Binding of the ligand stabilizes the protein, which increases the T_m-value. As shown in Table 1 all our dd-2′,3′-CDNs stabilize hSTING. Natural 2′,3′-cGAMP generated the largest stabilization (ΔT_m) by 16.2 °C, followed by dd-2′,3′-cGAMP (**1**), which stabilized by 13.1 °C. This shows that the OH groups indeed influence the binding to STING but that they are not essential. In contrast dd-2′,3′-cAAMP (**2**) and dd-2′,3′-cAGMP (**3**) showed a significantly smaller stabilization effect of $\Delta T_m = 2$ °C. For dd-2′,3′-cAAMP (**2**) this is less than half compared to the OH-containing reference compound 2′,3′-cAAMP with $\Delta T_m = 5.5$ °C. These data suggest that the nucleoside exchange from G to A has a much more dramatic influence on binding than the OH groups.

We next performed isothermal titration calorimetry (ITC) to gain deeper insight into the binding event (Table 1). Indeed, the lack of the two OH groups reduced the affinity, but binding was still observed in the submicromolar regime. For dd-2′,3′-cGAMP (**1**) we determined a K_D of 445 nM, mostly because of a loss of enthalpic stabilization. dd-2′,3′-cAAMP (**2**) showed reduced binding compared

Table 1: EC$_{50}$ and affinity data of dd-2′,3′-CDNs **1**–**3**.[a]

Compound	EC$_{50}$ [μM][b]	ΔT_m [°C] (nDSF)[c]	K_D [μM] (ITC)[d]
dd-2′,3′-cGAMP (**1**)	7.4 ± 1.7	13.1	0.45 ± 0.23
dd-2′,3′-cAAMP (**2**)	74.4 ± 4.6	1.90 ± 0.04	15.1 ± 14.5
dd-2′,3′-cAGMP (**3**)	>110	2.30 ± 0.02	42.9 ± 35.1
2′,3′-cAAMP	26.6 ± 4.9	5.50 ± 0.01	4.98 ± 0.82
2′,3′-cGAMP	10.6[e]	16.2 ± 0.1	0.004[f]

[a] Green: Synthesized dd-2′,3′-CDNs **1**–**3**. Blue: OH-containing reference compounds. [b] EC$_{50}$ values were measured in THP-1-Dual monocytic cells in three independent experiments. [c] Thermal shift temperatures are obtained from nDSF experiments. The temperature represents the difference in melting temperature between 5 μM hSTING incubated with 100 μM of the respective ligand and 5 μM hSTING without ligand. The results are mean values from three independent experiments. [d] K_D values are calculated from ITC experiments with an error from the individual fit of the binding model to the experimental data. [e] A single-replicate experiment was conducted to confirm literature known EC$_{50}$ values of 2′,3′-cGAMP.[27] [f] K_D value published by Zhang et al.[10]
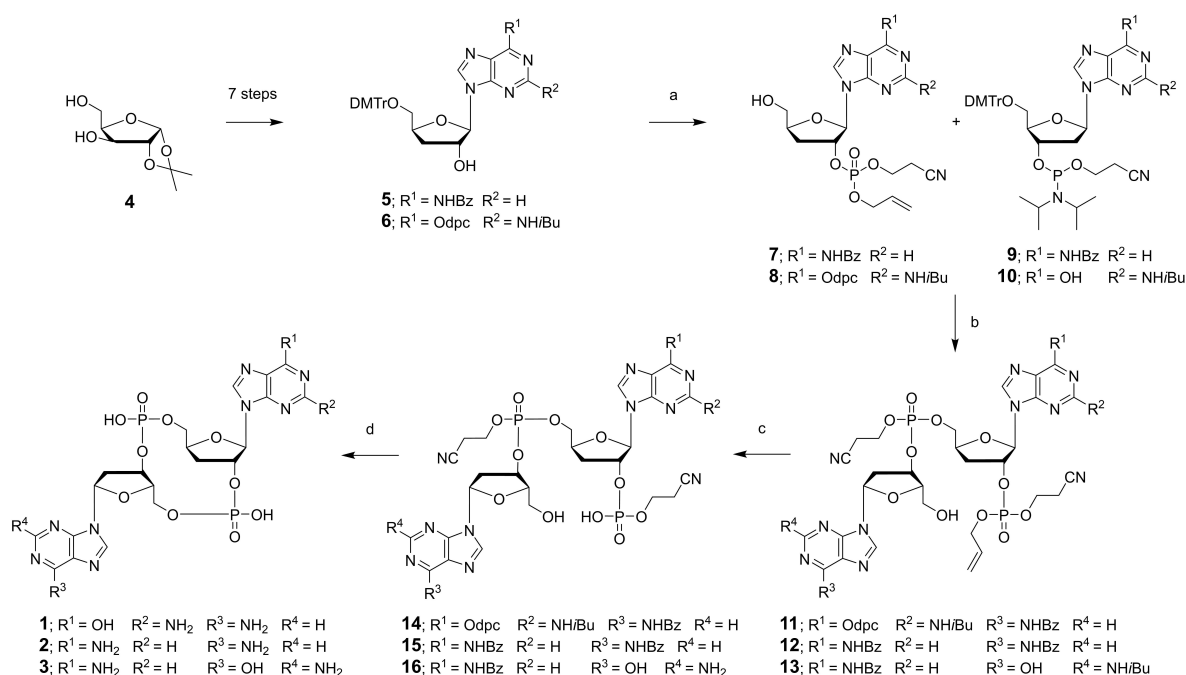
Scheme 1. Synthesis of dd-2′,3′-cGAMP derivatives **1**, **2** and **3**. Conditions: a) 1. 2-Cyanoethyl N,N,N′,N′-tetraisopropylphosphorodiamidite, pyridine trifluoroacetate, DCM, RT, 3 h; 2. BTT, allyl alcohol, RT, 1 h; 3. tBuOOH, RT, 40 min; 4. 3% v/v DCA in DCM, RT, 15 min; b) 1. BTT, MeCN, RT, 1 h; 2. tBuOOH, RT, 40 min; 3. 3% v/v DCA in DCM, RT, 10 min; c) NaI in acetone, reflux, 3 h; d) 1. MSNT, pyridine, RT, 18 h; 2. 33% v/v MeNH$_2$ in EtOH, RT, 4 h.

to reference compound 2′,3′-cAAMP by a factor of 3, while for dd-2′,3′-cAGMP (**3**) binding shifted to a higher micromolar value. Most importantly, all compounds, particularly dd-2′,3′-cGAMP (**1**) with a K_D of 0.5 μM, have a higher binding affinity to STING compared to other recently reported nucleoside agonists.[26]

To investigate the in cellulo activity of the prepared dd-2′,3′-CDNs **1**, **2** and **3**, we next measured their ability to induce an interferon response in immune cells (Table 1). For this purpose, we used a THP-1 monocytic reporter cell line containing a Lucia luciferase gene under the control of a promoter that is stimulated by 5 IFN-stimulated response elements. This allows to study the activation of the interferon pathway by measuring luminescence intensities. For control studies we used a THP-1 reporter cell line with STING being knocked out (STING-KO). For dd-2′,3′-cGAMP (**1**) and dd-2′,3′-cAAMP (**2**) ($c = 200$–300 μM, 37 °C, 24 h) we did not detect luminescence in the STING-KO control cell line, showing that both compounds operate as expected in a strictly STING-dependent fashion. In contrast, STING-competent THP-1 cells showed a strong luminescence response upon 2′,3′-cGAMP treatment. Concentration-dependent studies allowed us to determine an EC$_{50}$ of 10.6 μM for 2′,3′-cGAMP, which is in good agreement with literature data.[27] When performing the measurements using dd-2′,3′-cGAMP (**1**) we again determined a strictly STING-dependent response with an EC$_{50}$ of 7.4 μM, which is even slightly lower compared to natural 2′,3′-cGAMP itself. This is surprising given that the lack of 3′-OH groups reduces the affinity to STING. One possible explanation could be a different cellular uptake triggered by the lacking two OH

groups. For reference compound 2′,3′-cAAMP we measured an EC$_{50}$ of 27 μM and for dd-2′,3′-cAAMP (**2**) an EC$_{50}$ of 74 μM was determined. The dd-2′,3′-cAGMP (**3**) derivative gave an EC$_{50}$ of >110 μM. These results show that all dideoxy compounds show in cellulo STING activation. In the case of dd-2′,3′-cGAMP (**1**) it goes even beyond the capability of parent compound 2′,3′-cGAMP.

To test the stability of dd-2′,3′-CDNs **1** and **2** towards poxin degradation and compare it with 2′,3′-cGAMP, BHK-21 cells were infected with vaccinia virus WR ($\approx 1 \times 10^7$ PFUs) for one hour and subsequently incubated for another 14 hours to allow expression of viral particles and poxin enzymes. Cell lysates were prepared in a lysis buffer providing suitable conditions for maintaining the enzymatic activity of the poxins.[9] After removal of cell debris and protein quantification, 30 ng of total protein lysate per sample were incubated either with 2′,3′-cGAMP or with compounds **1** and **2** for up to 24 hours, thereby establishing a time course experiment with increasing sampling time intervals. Upon sampling, enzymes were inactivated by addition of a phenol: chloroform mixture (1:1). The aqueous fractions of the samples were purified and subsequently analyzed by LC–MS. Here, all compounds were unequivocally identified via their exact mass and quantified via their UV absorption at 260 nm. The resulting data is depicted in Figure 2. Our data show that the OH- containing reference compound 2′,3′-cGAMP is quickly hydrolyzed by the viral poxins. This is not the case for the dideoxy compounds. Indeed, our data show that dd-2′,3′-CDNs **1** and **2** are stable towards poxin degradation and therefore able to evade the viral degradation response. Taken together, dd-
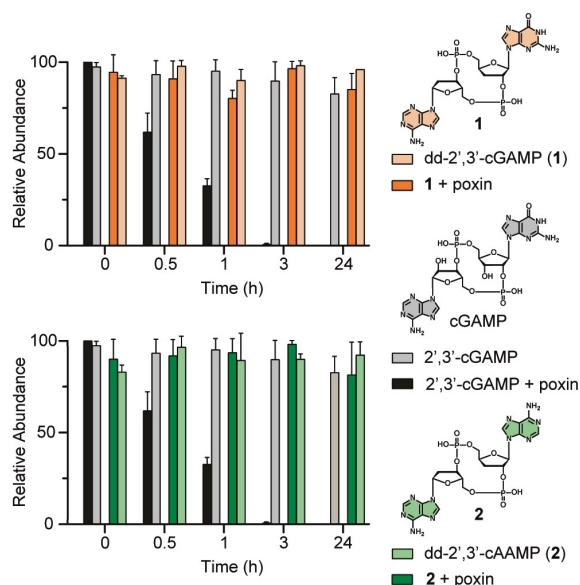
Figure 2. Stability of the dd-2′,3′-CDNs **1** and **2** and of the reference compound 2′,3′-cGAMP against poxin catalyzed degradation. ■=**1**, ■=**1**+poxin; ■=2′,3′-cGAMP, ■=2′,3′-cGAMP+poxin; ■=**2**, ■=**2**+poxin. All values are normalized to the amount of compound present at $t=0$ h, which was set to 100%. Error bars represent the standard deviation of three independent experimental replicates.
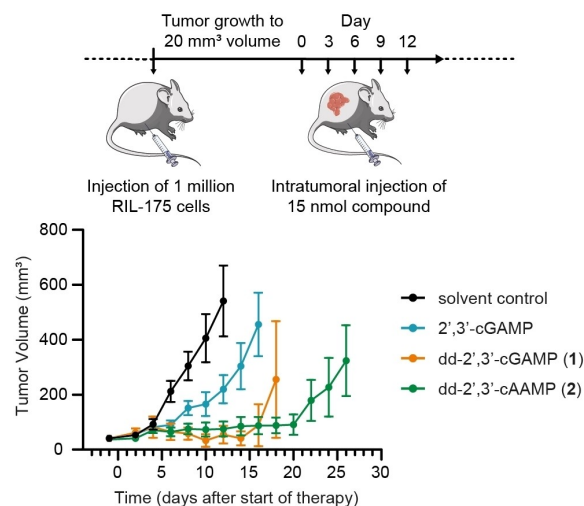

Figure 3. Schematic presentation of the in vivo xenograft tumor mouse model and the in vivo data, which show a dramatic control of the tumor growth particularly with dd-2′,3′-cAAMP (**2**): Top: Treatment scheme. Mice were subcutaneously inoculated with RIL-175 tumor cells (hepatocellular carcinoma cells). Once tumors reached a mean volume of 20 mm^3, intratumoral therapy on every third day was initiated. Solvent control or 15 nmol of 2′,3′-cGAMP, dd-2′,3′-cGAMP (**1**) or dd-2′,3′-cAAMP (**2**) were used for up to five treatments as depicted. Bottom: Tumor growth of CDN-treated tumors ($n=11$ for solvent control, $n=12$ for 2′,3′-cGAMP and dd-2′,3′-cAAMP (**2**) and $n=6$ for dd-2′,3′-cGAMP (**1**)). Mean tumor volume $+/-$ SEM is shown. Pooled data from two independent experiments are shown.

2′,3′-cGAMP (**1**) and dd-2′,3′-cAAMP (**2**) are stable STING agonists with reduced affinity but with remarkable EC$_{50}$ values.

In order to finally clarify how compounds **1** and **2** would behave in a tumor model, we examined their therapeutic efficacy in a mouse model of hepatocellular carcinoma (HCC) targeting mouse STING (mSTING). Analysis of published crystal structures and active site sequences shows that the interaction of mSTING and hSTING with the 3′-OH group should be similar.[7,28] For the study, 1×10^6 RIL-175 tumor cells were subcutaneously injected into C57BL/6 mice. The mice were treated five times by intratumoral injections of solvent control ($n=11$), 2′,3′-cGAMP ($n=12$), dd-2′,3′-cAAMP (**2**) ($n=12$) and dd-2′,3′-cGAMP (**1**) ($n=6$). The data together with a schematic representation of the experiment are shown in Figure 3. The data show that intratumoral injection of 2′,3′-cGAMP into RIL-175 tumors led to a significant delay in tumor growth (Figure 3 and Figure SI-5). Unexpectedly, we observed with dd-2′,3′-CDNs **1** and **2** a superior delay in tumor growth compared to parent compound 2′,3′-cGAMP (Figure 3 and Figure SI-5). At this point we believe that one reason for the better in vivo effect could be an improved cellular uptake of the dideoxy compounds as already hypothesized for the exceptional EC$_{50}$ values. In addition, the "less is more" paradigm could be at work here, which argues that a lower affinity of the compounds to the STING adaptor protein could result in a decreased T-cell toxicity, which has been described for high concentration of STING agonists.[29,30] Certainly, the surprisingly high EC$_{50}$ values and the strong in vivo tumor growth control require deeper mechanistic investigation.

In conclusion, we described the synthesis of 2′,3′-cGAMP dideoxy derivatives with superior in vivo characteristics for potential use as anti-viral and anti-tumoral therapeutics. The 2′- and 3′-OH groups of 2′,3′-cGAMP, which is the natural ligand for the adaptor protein STING, have been discussed as key elements that allow STING binding and enable STING to differentiate the 2′,3′-linked cGAMP derivative from 3′,3′-linked cyclic dinucleotides, of which the latter are key bacterial second messengers. The OH groups do affect binding of the ligands in two ways: first the 3′-OH group is known to establish a H-bond with Ser162 of the protein; second, the OH groups change and define the pucker of the ribose unit.[10] In deoxyribonucleotides it is known that the ribose can exist both in the C2′-endo and C3′-endo conformation, while for the ribonucleotides containing a free 2′-OH groups, a clear conformational preference for the C3′-endo conformation is reported as needed for binding.[31] The synthesis of the key compounds dd-2′,3′-cGAMP (**1**) and of dd-2′,3′-cAAMP (**2**) were possible using a new and concise synthetic combination of phosphoramidite and phosphotriester chemistry. ITC measurements show that the OH groups have only a minor enthalpic effect, but they make binding entropically more unfavorable because of the less preorganized structure of the ribose unit (Figure SI-3 and Table SI-1). Despite this effect, all prepared dideoxy-compounds show specific STING binding and STING pathway activation. Due to the lack of the 2′-OH groups, which is exploited by poxins to initiate 2′,3′-cGAMP hydrolysis, our compounds resist poxin degradation. Unexpectedly, we

observe in a preclinical tumor model that particularly dd-2′,3′-cAAMP (**1**) is able to control the tumor growth far better than the parent compound and natural ligand 2′,3′-cGAMP.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

[1] X. Tan, L. Sun, J. Chen, Z. J. Chen, *Annu. Rev. Microbiol.* **2018**, *72*, 447–478.

[2] G. P. Amarante-Mendes, S. Adjemian, L. M. Branco, L. C. Zanetti, R. Weinlich, K. R. Bortoluci, *Front. Immunol.* **2019**, *9*, 2379.

[3] K. N. Miller, S. G. Victorelli, H. Salmonowicz, N. Dasgupta, T. Liu, J. F. Passos, P. D. Adams, *Cell* **2021**, *184*, 5506–5526.

[4] A. Ablasser, M. Goldeck, T. Cavlar, T. Deimling, G. Witte, I. Röhl, K.-P. Hopfner, J. Ludwig, V. Hornung, *Nature* **2013**, *498*, 380–384.

[5] L. Sun, J. Wu, F. Du, X. Chen, Z. J. Chen, *Science* **2013**, *339*, 786–791.

[6] J. Wu, L. Sun, X. Chen, F. Du, H. Shi, C. Chen, Z. J. Chen, *Science* **2013**, *339*, 826–830.

[7] P. Gao, M. Ascano, Y. Wu, W. Barchet, B. L. Gaffney, T. Zillinger, A. A. Serganov, Y. Liu, R. A. Jones, G. Hartmann, T. Tuschl, D. J. Patel, *Cell* **2013**, *153*, 1094–1107.

[8] C. Zhang, G. Shang, X. Gui, X. Zhang, X.-C. Bai, Z. J. Chen, *Nature* **2019**, *567*, 394–398.

[9] J. B. Eaglesham, Y. Pan, T. S. Kupper, P. J. Kranzusch, *Nature* **2019**, *566*, 259–263.

[10] X. Zhang, H. Shi, J. Wu, X. Zhang, L. Sun, C. Chen, Z. J. Chen, *Mol. Cell* **2013**, *51*, 226–235.

[11] D. Gao, J. Wu, Y.-T. Wu, F. Du, C. Aroh, N. Yan, L. Sun, J. Chen Zhijian, *Science* **2013**, *341*, 903–906.

[12] M. Moriyama, T. Koshiba, T. Ichinohe, *Nat. Commun.* **2019**, *10*, 4624.

[13] X.-D. Li, J. Wu, D. Gao, H. Wang, L. Sun, J. Chen Zhijian, *Science* **2013**, *341*, 1390–1394.

[14] J. Paijo, M. Döring, J. Spanier, E. Grabski, M. Nooruzzaman, T. Schmidt, G. Witte, M. Messerle, V. Hornung, V. Kaever, U. Kalinke, *PLoS Pathog.* **2016**, *12*, e1005546.

[15] B. Sun, K. B. Sundström, J. J. Chew, P. Bist, E. S. Gan, H. C. Tan, K. C. Goh, T. Chawla, C. K. Tang, E. E. Ooi, *Sci. Rep.* **2017**, *7*, 3594.

[16] Y. Wang, J. Luo, A. Alu, X. Han, Y. Wei, X. Wei, *Mol. Cancer* **2020**, *19*, 136.

[17] L. T. Khoo, L.-Y. Chen, *EMBO Rep.* **2018**, *19*, e46935.

[18] J. Kwon, S. F. Bakhoum, *Cancer Discovery* **2020**, *10*, 26–39.

[19] A. Amouzegar, M. Chelvanambi, J. N. Filderman, W. J. Storkus, J. J. Luke, *Cancers* **2021**, *13*, 2695.

[20] D.-S. Kim, A. Endo, F. G. Fang, K.-C. Huang, X. Bao, H.-W. Choi, U. Majumder, Y. Y. Shen, S. Mathieu, X. Zhu, K. Sanders, T. Noland, M.-H. Hao, Y. Chen, J. Y. Wang, S. Yasui, K. TenDyke, J. Wu, C. Ingersoll, K. A. Loiacono, J. E. Hutz, N. Sarwar, *ChemMedChem* **2021**, *16*, 1741–1744.

[21] W. Chang, M. D. Altman, C. A. Lesburg, S. A. Perera, J. A. Piesvaux, G. K. Schroeder, D. F. Wyss, S. Cemerski, Y. Chen, E. DiNunzio, A. M. Haidle, T. Ho, I. Kariv, I. Knemeyer, J. E. Kopinja, B. M. Lacey, J. Laskey, J. Lim, B. J. Long, Y. Ma, M. L. Maddess, B.-S. Pan, J. P. Presland, E. Spooner, D. Steinhuebel, Q. Truong, Z. Zhang, J. Fu, G. H. Addona, A. B. Northrup, E. Parmee, J. R. Tata, D. J. Bennett, J. N. Cumming, T. Siu, B. W. Trotter, *J. Med. Chem.* **2022**, *65*, 5675–5689.

[22] J. Le Naour, L. Zitvogel, L. Galluzzi, E. Vacchelli, G. Kroemer, *Oncoimmunology* **2020**, *9*, 1777624–1777624.

[23] F. Hulpia, S. Van Calenbergh, G. Caljon, L. Maes, Universiteit Gent, Universiteit Antwerpen, WO2019076633, **2019**.

[24] A. Kumar, S. I. Khan, A. Manglani, Z. K. Khan, S. B. Katti, *Nucleosides Nucleotides Nucleic Acids* **1994**, *13*, 1049–1058.

[25] C. Meier, J. M. Neumann, F. Andre, Y. Henin, T. Huynh Dinh, *J. Org. Chem.* **1992**, *57*, 7300–7308.

[26] C. Ding, Z. Song, A. Shen, T. Chen, A. Zhang, *Acta Pharm. Sin. B* **2020**, *10*, 2272–2298.

[27] B. Novotná, L. Vaneková, M. Zavřel, M. Buděšínský, M. Dejmek, M. Smola, O. Gutten, Z. A. Tehrani, M. Pimková Polidarová, A. Brázdová, R. Liboska, I. Štěpánek, Z. Vavřina, T. Janduší́k, R. Nencka, L. Rulíšek, E. Bouřa, J. Brynda, O. Páv, G. Birkuš, *J. Med. Chem.* **2019**, *62*, 10676–10690.

[28] D. Lu, G. Shang, J. Li, Y. Lu, X.-C. Bai, X. Zhang, *Nature* **2022**, *604*, 557–562.

[29] K. E. Sivick, A. L. Desbien, L. H. Glickman, G. L. Reiner, L. Corrales, N. H. Surh, T. E. Hudson, U. T. Vu, B. J. Francica, T. Banda, G. E. Katibah, D. B. Kanne, J. J. Leong, K. Metchette, J. R. Bruml, C. O. Ndubaku, J. M. McKenna, Y. Feng, L. Zheng, S. L. Bender, C. Y. Cho, M. L. Leong, A. van Elsas, T. W. Dubensky, S. M. McWhirter, *Cell Rep.* **2018**, *25*, 3074–3085.

[30] M. F. Gulen, U. Koch, S. M. Haag, F. Schuler, L. Apetoh, A. Villunger, F. Radtke, A. Ablasser, *Nat. Commun.* **2017**, *8*, 427.

[31] W. Saenger, *Principles of Nucleic Acid Structure*, Springer, New York, **1984**.