



HAL
open science

Development of 2-deoxystreptamine–nucleobase conjugates for the inhibition of oncogenic miRNA production

Thi Phuong Anh Tran, Sylvain Poulet, Mélanie Pernak, Anita Rayar, Stéphane Azoulay, Audrey Di Giorgio, Maria Duca

► **To cite this version:**

Thi Phuong Anh Tran, Sylvain Poulet, Mélanie Pernak, Anita Rayar, Stéphane Azoulay, et al.. Development of 2-deoxystreptamine–nucleobase conjugates for the inhibition of oncogenic miRNA production. RSC Medicinal Chemistry, 2022, 13 (3), pp.311-319. 10.1039/D1MD00345C . hal-03852423

HAL Id: hal-03852423

<https://hal.science/hal-03852423>

Submitted on 15 Nov 2022

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

1 **Development of 2-deoxystreptamine-nucleobase conjugates for the inhibition of oncogenic**
2 **miRNAs production**

3

4 Thi Phuong Anh Tran,^{‡,a} Sylvain Poulet,^a Mélanie Pernak,^a Anita Rayar,^a Stéphane Azoulay,^a Audrey
5 Di Giorgio,^a Maria Duca^{*,a}

6

7 ^a Université Côte d'Azur, CNRS, Institut de Chimie de Nice (ICN), Nice, France

8 *Corresponding author: maria.duca@univ-cotedazur.fr

9 [‡] Present address: Department of Chemistry Engineering, Nha Trang University, 02 Nguyen Dinh Chieu,
10 Nha Trang, Khanh Hoa, Viet Nam

11

12

1 **Abstract**

2 The discovery of new original scaffolds for selective RNA targeting is one of the main challenges of
3 current medicinal chemistry since therapeutically relevant RNAs represent potential targets for a number
4 of pathologies. Recent efforts have been devoted to the search for RNA ligands targeting the biogenesis
5 of oncogenic miRNAs whose overexpression has been directly linked to the development of various
6 cancers. In this work, we developed a new series of RNA ligands for the targeting of oncogenic miRNAs
7 biogenesis based on the 2-deoxystreptamine scaffold. This latter is part of the aminoglycoside neomycin
8 and is known to play an essential role in the RNA interaction of this class of RNA binders. 2-
9 deoxystreptamine was thus conjugated to natural and artificial nucleobases to obtain new binders of
10 oncogenic miR-372 precursor (pre-miR-372). We identified some conjugates bearing a similar
11 biological activity compared to previously synthesized neomycin analogs and studied their mode of
12 binding with the target pre-miR-372.

13

14

1 **Introduction**

2 Targeting RNA using specific small molecules represents one of the great challenges of current
3 medicinal chemistry and the first clinical successes of this paradigm are already on the market.^{1,2} First
4 of all, a number of antibiotics targeting prokaryotic ribosomal RNA have been employed in therapy for
5 many decades.³ Furthermore, a recent breakthrough has been made with the commercialization in 2020
6 of Risdiplam as a modifier of RNA splicing in spinal muscular atrophy (SMA).⁴ This compound acts
7 upon binding to the SMN2 mRNA inducing the correction of splicing errors causing the deadly
8 pathology and represents a major progress for treatment. Various therapeutically relevant RNAs are
9 currently under study as innovative and original targets, such as viral RNAs and oncogenic RNAs.⁵ In
10 this last class, microRNAs (miRNAs or miRs) are of particular interest as major regulators of gene
11 expression. MicroRNAs are short non-coding RNAs that recognize mRNAs and control protein
12 expression upon inducing their degradation.⁶ Each miRNA is responsible for the regulation of hundreds
13 of proteins thus being part of a massive regulation process inside cells. Despite being essential, miRNAs
14 can be deregulated (overexpressed or underexpressed) and variations in the expression of miRNAs have
15 been directly correlated with various pathologies such as cancer.⁷ For this reason, miRNAs have been
16 studied as potential therapeutic targets.⁸

17 Various strategies have been developed in particular to tackle the overexpression of oncogenic miRNAs.
18 Oligonucleotides can directly inhibit the function of oncogenic miRNAs upon complementary
19 hybridization.⁹ This specific approach is particularly successful, but still bears some limitations
20 regarding its clinical application.¹⁰ Small molecules were also developed to inhibit the biogenesis of
21 oncogenic miRNAs upon binding to oncogenic miRNAs precursors such as primary miRNAs (pri-
22 miRNAs) and pre-miRNAs.¹¹ This approach is based on the search for RNA binders selective for a
23 particular secondary and/or tertiary structure present in the precursors of the targeted miRNA that are
24 called pre-miRNAs and pri-miRNAs and that bear structures suitable for small-molecule binding.
25 Among the most successful examples of this strategy, compounds from the group of Disney designed
26 using the Inforna approach demonstrated to be very specific inhibitors of miRNAs biogenesis.¹²⁻¹⁵
27 Screening of compound libraries containing a collection of RNA binders also gave interesting results.¹⁶⁻
28 ²⁰ Our group contributed to the field with the design of multimodal RNA ligands synthesized upon
29 conjugation of various RNA binding domains bringing both affinity and selectivity for the target.²¹⁻²⁴
30 More specifically, we built conjugates, whose general structure is illustrated in Figure 1A, between
31 aminoglycosides (such as neomycin) that are strong but non-selective RNA ligands with natural and
32 artificial nucleobases that are heteroaromatic compounds able to selectively recognize RNA bases and
33 RNA base pairs, respectively.²⁵

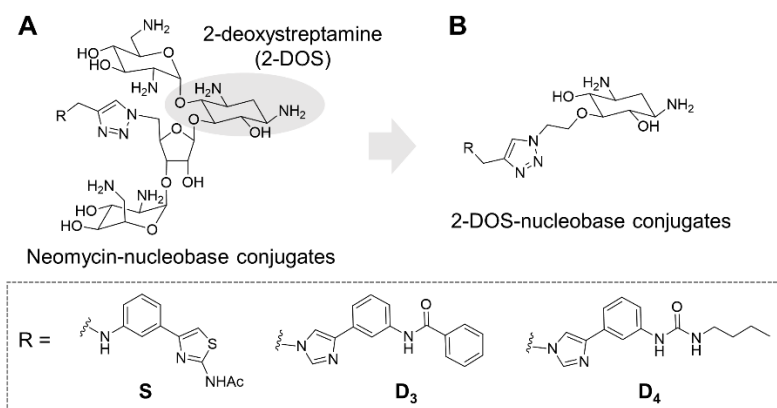


Figure 1. General structure of previously synthesized neomycin-nucleobase conjugates (A) and of the newly designed 2-DOS-nucleobase conjugates (B). The detailed structure for nucleobases **S**, **D₃** and **D₄** is illustrated in the dashed square.

This led us to the identification of RNA ligands inducing the inhibition of the production of an oncogenic miRNA, miR-372, *in vitro* and in cancer cells overexpressing this miRNA. Some of the synthesized compounds were able to affect the biogenesis of a small set of miRNAs sharing common precursors or common binding sites with pre-miR-372 and, importantly, sharing the same protein targets. The main issue with these aminoglycoside conjugates is the size and the hydrophilicity of the obtained compounds. Both these features limit the perspectives for clinical applications because the physico-chemical properties are not drug-like and because they probably contribute to the lack of specificity. The analysis of aminoglycoside structures reveals that the vast majority contains the meso-1,3 diaminocyclitol 2-deoxystreptamine (2-DOS, Figure 1A), glycosylated at the 4-position, as well as the 5- (neomycin class) or 6- (kanamycin-gentamicin class) position with a variety of aminosaccharides.²⁶ In this work, we thus decided to replace the neomycin moiety with 2-deoxystreptamine, which is part of neomycin and is known to play an important role in the RNA binding ability of aminoglycosides. 2-DOS has been reported to bind weakly to two base units within a disrupted RNA helix. Solution studies carried out with 2-DOS have shown that it binds to 5'-3' two-base steps (including GU, UG, and GG), albeit with a very low affinity (>1 mM).²⁷ For this reason, various conjugates of 2-DOS have been previously reported as dimers for prokaryotic ribosomal RNA binding but also for the inhibition of miRNA maturation.²⁸⁻³⁰ Here, we report the synthesis of a series of 2-DOS conjugates (Figure 1B) analogs of a previous neomycin-conjugated series in order to study their biological activities and structure-activity relationships in the context of the inhibition of miR-372 biogenesis. We found that some conjugates bear a very good affinity for pre-miR-372 target and that, although the affinity is lower than the one of their neomycin counterpart, the inhibition activity is maintained. After the evaluation of binding affinity, selectivity, inhibition activity and site of interaction, we identified one compound with very promising binding and inhibition properties compared to its neomycin counterpart and bearing much better physico-chemical properties for future development.

1 **Methods**

2 **Chemistry**

3 Reagents and solvents were purchased from Merck or Carlo Erba and used without further purification.
4 All reactions that involved air- or moisture-sensitive reagents or intermediates were performed under
5 argon atmosphere. Flash column chromatography was carried out on silica gel (Merck; SDS 60 Å, 40-
6 63 µM, VWR). Analytical TLC was conducted on pre-coated silica gel plates (60F254; Merck) and
7 compounds were visualized by irradiation ($\lambda = 254$ nm) or by staining with ninhydrin stain. ^1H and ^{13}C
8 NMR spectra were recorded on Bruker AC 200, 400 and 500 MHz spectrometers. Chemical shifts are
9 reported in parts per million (ppm, δ) referenced to the residual ^1H resonance of the solvent (CDCl_3 δ
10 7.26; CD_3OD δ 3.31; $\text{DMSO}-d_6$ δ 2.50; $\text{acetone}-d_6$ δ 2.05 ppm). Splitting patterns are designed as
11 follows: s (singlet), d (doublet), t (triplet), m (multiplet), and br (broad). Coupling constant (J) are listed
12 in hertz (Hz). High-resolution mass spectrometry (HRMS) was carried out on a LTQ Orbitrap hybrid
13 mass spectrometer with an electrospray ionization probe (ThermoScientific, San Jose, CA) by direct
14 infusion from a pump syringe, to confirm the correct molar mass and high purity of the compounds.
15 HPLC analyses were performed using a Water ARC UHPLC pump coupled to a Water 2998 photodiode
16 array detector and Waters Cortex® C18+ column (50 x 4.6 mm, 2.7 µm). Analysis were run out at room
17 temperature by using a gradient of CH_3CN containing 0.1% TFA (eluent B) in water containing 0.1%
18 TFA (eluent A) at a flow rate of 1.5 mL/min. The employed gradient was: 5 → 40% eluent B over 5
19 min, and 40 → 100 % eluent B over 2 min.

20 **Synthetic procedures**

21 The preparation of Boc-protected 2-DOS derivatives **1**, **2**, **3** and **4a-h** as well as the synthesis of modified
22 nucleobases containing the alkyne chain **10a-h** are described in the Supporting Information.

23 **General procedure for the 1,3-dipolar cycloaddition (General procedure A).** 1,3-Dipolar
24 cycloaddition was performed starting from the azido derivative of 2-DOS **3** and appropriately alkyne-
25 modified nucleobases in order to obtain desired 1,2,3-triazole derivative of 2-DOS. To a solution **3** or **7**
26 (100 mg, 0.196 mmol) and alkynes **10a-h** (0.216 mmol, 1.1 eq.) in CH_3CN (11 mL), CuI (74.5 mg, 0.4
27 mmol, 2eq.) and DIPEA (205 µL, 1.18 mmol) were added and the reaction mixture was stirred at room
28 temperature or under reflux for the reported times. The solvent was then removed under reduced pressure
29 and the crude residue was purified by flash chromatography on a silica gel column leading to the desired
30 1,4-disubstituted 1,2,3-triazole as a white solid.

31 **General procedure for deprotection of Boc and acetal groups (General procedure B)**

32 To a solution of protected compounds **4a-h** or **8** in CH_2Cl_2 and H_2O (3/1). TFA (50 eq.) was then added
33 and the reaction mixture was stirred at room temperature overnight. The solvent and the residues of TFA
34 were then removed under reduced pressure. Precipitation in ethyl ether led to pure compounds as white

1 solid (TFA salts). Final removal of copper was performed upon stirring 1h at r.t. in the presence of
2 Chelex resin.

3 **2-deoxystreptamine-4-O-[adenyl-N-ethyl]triazol (5a)**. General procedure **B** was applied to solution
4 of **4a** (100 mg, 0.146 mmol) in CH₂Cl₂ (3 mL) and water (1 mL) in the presence of TFA (557 μL, 7.32
5 mmol). Pure compound **5a** was obtained after precipitation in CH₂Cl₂ and Chelex resin treatment as a
6 white solid in 60% yield (55.3 mg). Retention time 0.91 min; ¹H NMR (500 MHz, CD₃OD) δ ppm: 8.30-
7 8.10 (m, 3H), 5.63 (br s, 2H), 4.65 (s, 2H), 4.45-4.50 (m, 1H), 4.15-4.10 (m, 1H), 3.39-3.20 (m, 4H),
8 3.15-3.10 (m, 1H), 2.40-2.30 (m, 1H), 1.55-1.45 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ ppm: 163.1,
9 162.8, 156.7, 143.1, 140.3, 129.9, 126.9, 82.5, 77.2, 74.5, 71.7, 55.8, 51.9, 51.4, 50.8, 29.8; MS (ESI)
10 *m/z* 405.21063 [M+H]⁺ (C₁₆H₂₅O₃N₁₀ requires 405.21056).

11 **2-deoxystreptamine-4-O-[uracil-N-ethyl]triazol (5b)**. General procedure **B** was applied to a solution
12 of **4b** (27 mg, 0.0408 mmol) in CH₂Cl₂ (0.6 mL) and water (0.2 mL) in the presence of TFA (149 μL,
13 2.04 mmol). Pure compound **5b** was obtained after precipitation in CH₂Cl₂ and Chelex resin treatment
14 as a white solid in 98% yield (24.3 mg). Retention time 0.82 min; ¹H NMR (500 MHz, CD₃OD) δ ppm:
15 8.06 (s, 1H), 7.72 (d, *J* = 3.2 Hz, 1H), 5.68 (d, *J* = 3.2 Hz, 1H), 5.02 (s, 2H), 4.64 (t, *J* = 2.2 Hz, 2H),
16 4.50-4.45 (m, 1H), 4.15-4.10 (m, 1H), 3.40-3.35 (m, 4H), 3.20-3.10 (m, 1H), 2.40-2.30 (m, 1H), 1.80-
17 1.70 (m, 1H); ¹³C NMR (125 MHz, CD₃OD) δ ppm: 166.9, 153.1, 147.4, 126.5, 103.1, 83.0, 77.7, 75.0,
18 72.3, 52.2, 51.8, 51.2, 44.6, 30.3; MS (ESI) *m/z* 382.18375 [M+H]⁺ (C₁₅H₂₄O₅N₇ requires 382.18334).

19 **2-deoxystreptamine-4-O-[cytosyl-N-ethyl]triazol (5c)**. General procedure **B** was applied to a solution
20 of **4c** (25.5 mg, 0.0385 mmol) in CH₂Cl₂ (0.6 mL) and water (0.3 mL) in the presence of TFA (149 μL,
21 1.93 mmol). Pure compound **5c** was obtained after precipitation in CH₂Cl₂ and Chelex resin treatment
22 as a white solid in 70% yield (10.2 mg). Retention time 0.76 min; ¹H NMR (500 MHz, CD₃OD) δ ppm:
23 8.17 (s, 1H), 8.04 (d, *J* = 3.1 Hz, 1H), 6.11 (br s, 1H), 5.13 (s, 2H), 4.67 (t, *J* = 2.2 Hz, 2H), 4.50-4.40
24 (m, 1H), 4.20-4.10 (m, 1H), 3.50-3.35 (m, 4H), 3.20-3.10 (m, 1H), 2.45-2.40 (m, 1H), 1.90-1.80 (m,
25 1H); ¹³C NMR (125 MHz, CD₃OD) δ ppm: 162.8, 150.9, 150.6, 126.5, 95.2, 82.4, 77.3, 74.5, 71.8, 51.8,
26 51.5, 50.9, 45.4, 29.8; MS (ESI) *m/z* 381.19952 [M+H]⁺ (C₁₅H₂₅O₄N₈ requires 381.19933).

27 **2-deoxystreptamine-4-O-[4-((3-benzamidophenyl)-1H-imidazol-1-yl)ethyl]triazol (5d)**. General
28 procedure **B** was applied to a solution of **4d** (60 mg, 0.0739 mmol) in CH₂Cl₂ (1.5 mL) and H₂O (0.5
29 mL) in the presence of TFA (854 μL, 3.69 mmol). Pure compound **5d** was obtained after precipitation
30 in CH₂Cl₂ and Chelex resin treatment as a white solid in 97% yield (55.0 mg). Retention time 2.78 min;
31 ¹H NMR (500 MHz, CD₃OD) δ ppm: 8.28 (s, 1H), 8.00-7.95 (m, 3H), 7.70-7.50 (m, 8H), 5.60 (s, 2H),
32 4.72 (t, *J* = 2.0 Hz, 2H), 4.50-4.45 (m, 1H), 4.20-4.15 (m, 1H), 3.45-3.35 (m, 4H), 3.15-3.10 (m, 1H),
33 2.45-2.40 (m, 1H), 1.80-1.75 (m, 1H); ¹³C NMR (125 MHz, CD₃OD) δ ppm: 169.5, 141.4, 136.4, 133.6,
34 131.4, 130.9, 130.1, 129.1, 123.5, 83.0, 77.7, 75.1, 73.5, 72.2, 49.9, 49.7, 49.6, 30.2; MS (ESI) *m/z*
35 533.26215 (C₂₇H₃₃O₄N₈ 533.26193).

36 **2-deoxystreptamine-4-O-[4-(3-aminophenyl)-1H-imidazol-1-yl)ethyl]triazol (5e)**. General
37 procedure **B** was applied to a solution of **4e** (49 mg, 0.0692 mmol) in CH₂Cl₂ (0.5 mL) and H₂O (0.2

1 mL) in the presence of TFA (265 μ L, 3.46 mmol). Pure compound **5e** was obtained after precipitation
2 in CH_2Cl_2 and Chelex resin treatment as a white solid in 97% yield (49.7 mg). Retention time 2.77 min;
3 ^1H NMR (400 MHz, CD_3OD) δ ppm: 9.08 (s, 1H), 8.27 (s, 1H), 7.90-7.80 (m, 2H), 7.39 (t, $J = 5.0$ Hz,
4 1H), 7.60-7.45 (m, 2H), 5.61 (s, 2H), 4.75-4.70 (m, 2H), 4.50-4.40 (m, 1H), 4.30-4.20 (m, 1H), 3.40-
5 3.30 (m, 3H), 3.22 (t, $J = 9.8$ Hz, 2H), 3.40-3.30 (m, 1H), 2.40-2.30 (m, 1H), 1.80-1.70 (m, 1H), 1.60-
6 1.50 (m, 2H), 1.40-1.30 (m, 2H), 0.97 (t, $J = 5.0$ Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 156.8,
7 143.1, 141.7, 140.1, 137.4, 134.3, 128.6, 118.7, 117.5, 115.6, 109.0, 98.4, 82.6, 77.2, 74.5, 71.8, 67.0,
8 51.4, 50.8, 49.6, 40.5, 33.3, 21.0, 14.1; MS (ESI) m/z 528.30426 ($\text{C}_{25}\text{H}_{38}\text{O}_4\text{N}_9$ 528.30413).

9 **2-deoxystreptamine-4-O-[4-(3-(2-naphthamido)phenyl)-1H-imidazol-1-yl]ethyl]triazol (5f).**
10 General procedure **B** was applied to a solution of **4f** (29 mg, 0.0336 mmol) in CH_2Cl_2 (0.5 mL) and H_2O
11 (0.2 mL) in the presence of TFA (129 μ L, 1.68 mmol). Pure compound **5f** was obtained after
12 precipitation in CH_2Cl_2 and Chelex resin treatment as a white solid in 87% yield (23.0 mg). Retention
13 time 2.45 min; ^1H NMR (400 MHz, CD_3OD) δ ppm: 8.54 (s, 1H), 8.29 (s, 1H), 8.20-7.90 (m, 5H), 7.75-
14 7.50 (m, 5H), 5.61 (s, 2H), 4.75-4.70 (m, 2H), 4.50-4.40 (m, 1H), 4.25-4.15 (m, 1H), 3.40-3.30 (m, 4H),
15 3.20-3.10 (m, 1H), 2.45-2.35 (m, 1H), 1.80-1.70 (m, 1H); ^{13}C NMR (100 MHz, CD_3OD) δ ppm: 141.0,
16 136.6, 133.2, 134.1, 133.2, 131.0, 130.1, 129.5, 129.3, 128.9, 128.0, 125.1, 122.8, 82.7, 77.3, 74.6, 71.8,
17 54.8, 51.8, 49.6, 31.3, 17.2.

18 **2-deoxystreptamine-4-O-[4-(3-pentanamidophenyl)-1H-imidazol-1-yl]ethyl]triazol (5g).** General
19 procedure **B** was applied to a solution of **4g** (30 mg, 0.0372 mmol) in CH_2Cl_2 (0.5 mL) and H_2O (0.2
20 mL) in the presence of TFA (143 μ L, 1.86 mmol). Pure compound **5g** was obtained after precipitation
21 in CH_2Cl_2 and Chelex resin treatment as a yellow solid in 87% yield (28.0 mg). Retention time 1.10
22 min; ^1H NMR (500 MHz, CD_3OD) δ ppm: 8.93 (s, 1H), 8.26 (s, 1H), 7.90 (s, 1H), 7.40-7.30 (m, 1H),
23 7.25-7.15 (m, 2H), 7.00-6.90 (m, 1H), 5.58 (s, 2H), 4.75-4.65 (m, 2H), 4.50-4.40 (m, 1H), 4.25-4.15 (m,
24 1H), 3.50-3.35 (m, 4H), 3.25-3.15 (m, 1H), 2.50-2.40 (m, 1H), 1.80-1.70 (m, 1H); ^{13}C NMR (125 MHz,
25 CD_3OD) δ ppm: 135.9, 130.1, 125.1, 118.5, 118.1, 117.4, 114.2, 81.2, 75.8, 73.1, 70.4, 50.4, 50.0, 49.4,
26 43.4, 28.4; MS (ESI) m/z 429.23608 ($\text{C}_{20}\text{H}_{29}\text{O}_3\text{N}_8$ 429.23571).

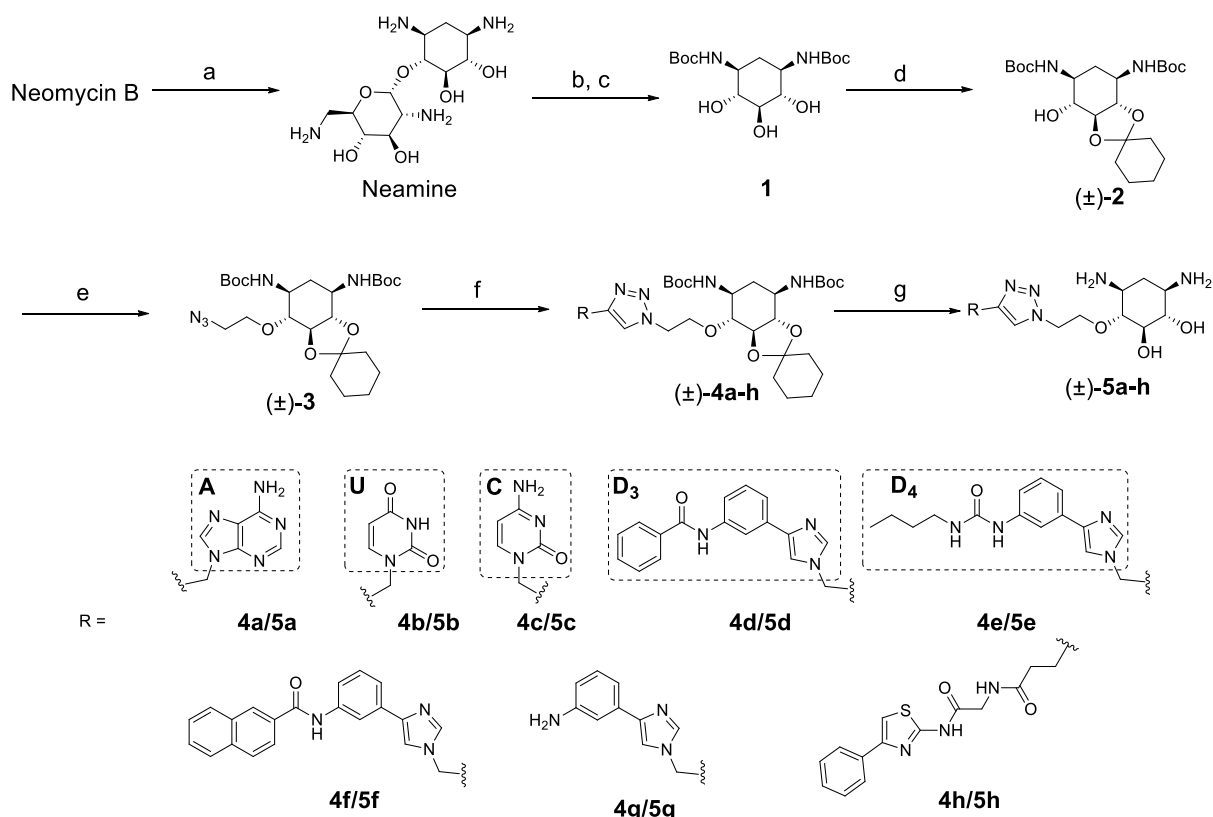
27 **1,3-Bis-*N*-(*tert*-butyloxycarbonyl)-5,6-*O*-cyclohexylidene-2-deoxystreptamine-4-*O*-[4-**
28 **phenylthiazol-2-yl]amino)butyl)-1H-imidazol-1-yl]ethyl]triazol (5h).** General procedure **B** was
29 applied to a solution of **4h** (16 mg, 0.033 mmol) in CH_2Cl_2 (0.5 mL) and water (0.2 mL) in the presence
30 of TFA (129 μ L, 1.65 mmol). Final compound **5h** was obtained as a white solid in 98% yield (12 mg).
31 Retention time min 10.5 min (Phenomenex Synergi 4 μm Fusion-RP 80 \AA column (250 x 4.6 mm); flow
32 rate of 1 mL/min, gradient 5 \rightarrow 40% eluent B over 20 min); ^1H NMR (400 MHz, MeOD) δ (ppm): 8.00-
33 7.85 (m, 3H), 7.40-7.30 (m, 3H), 7.30-7.20 (m, 1H), 4.60-4.50 (m, 2H), 4.50-4.40 (m, 1H), 4.20-4.10
34 (m, 3H), 3.50-3.35 (m, 4H), 3.20-3.00 (m, 3H), 2.70-2.60 (m, 2H), 2.40-2.30 (m, 1H), 1.80-1.70 (m,
35 1H); ^{13}C NMR (100MHz, CD_3OD) δ (ppm): 169.7, 159.1, 151.3, 135.8, 130.1, 129.5, 127.1, 108.8, 82.6,
36 77.3, 74.5, 72.1, 51.8, 51.4, 50.9, 43.4, 35.9, 30.0; MS (ESI) m/z 545.22974 ($\text{C}_{24}\text{H}_{33}\text{O}_5\text{N}_8\text{S}$ 545.22891).

1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28
29
30
31
32
33
34
35

Results and Discussion

Design of 2-DOS conjugates

The disubstituted 2-DOS-containing aminoglycosides represent a vast majority of this class of antibiotics and are strong RNA binders. The aim of this work is to assess if 2-DOS moiety could replace neomycin in our previously synthesized pre-miR-372 ligands.²³ These latter contained neomycin conjugated with natural and artificial nucleobases and some of them were efficient and selective inhibitors of oncogenic miR-372 maturation *in vitro* and in cells.^{23 25} Neomycin is known to bind to unpaired RNA bases at sites where the double helix is distorted by the presence of bulges or loops. Natural nucleobases should interact with unpaired bases *via* the formation of Watson-Crick-Franklin H-bonds and artificial nucleobases are meant to interact with paired bases *via* Hoogsteen H-bonds thus forming triplets.²⁵ So, the conjugation of neomycin to natural nucleobases would direct the compounds on single-stranded regions of bulges and loops, and the conjugation of neomycin to artificial nucleobase conjugates should direct the ligands close to stem-bulge and stem-loop junctions. In our previous studies, we observed that artificial nucleobase conjugates were more efficient and selective binders of pre-miR-372 than natural bases conjugates and some of them were able to inhibit its processing by Dicer enzyme *in vitro*.²³ A specific inhibition of proliferation of adenocarcinoma cancer cells (AGS) overexpressing miR-372 was also observed and this activity was linked to the inhibition of Dicer processing for miR-372. The study of the site of interaction of the best binders and inhibitors showed that they indeed bound at stem-bulge junctions and close to the site of cleavage of Dicer enzyme.^{21, 24} Even with these promising results in hand, as well as with a complete structure-activity characterization of this series of conjugates,^{21, 22, 24} the size of the obtained compounds as well as their high hydrophilicity represent relevant drawbacks for the physico-chemical properties of this kind of conjugates. Furthermore, neomycin is known to be a toxic drug when administered and to lack specificity when used against a particular RNA. We thus decided to prepare new analogs of these conjugates by replacing the neomycin moiety by its substructure 2-DOS and to study the activity of these compounds against miR-372 maturation that we took as a model for comparison with our previously reported neomycin conjugates. 2-DOS represents a key structural feature of most aminoglycosides and occupies a central position in their structure as well as an essential role for their interaction with prokaryotic ribosomal RNA. We thus wondered if the small 2-DOS moiety could be sufficient for binding yet leading to more favorable physico-chemical properties in the final conjugates. The most active compound in the neomycin series was the one containing nucleobase **S**.²³ However, the 2-DOS analog of this conjugate was previously reported and did not show activity for miR-372 maturation inhibition.²⁴ As illustrated in Scheme 1, we thus chose to conjugate 2-DOS with natural nucleobases adenine (A), cytosine (C) and uracil (U) that were not studied previously.



1
 2 **Scheme 1.** Synthesis of the new series of 2-DOS conjugates **5a-h**. Reagents: a) HCl, reflux, overnight;
 3 b) 48% HBr aq., reflux, 2 days; c) Boc₂O, NaOH, dioxane/H₂O, overnight; d) 1,1-
 4 dimethoxycyclohexane, pTsOH, DMF, 50°C, 55 mbar, 1h; e) 2-Azidoethyl-p-toluensulfonate, NaH,
 5 THF, 30°C, 3h; f) **10a-h**, CuI, DIPEA, CH₃CN, from r. t. to 50°C, from 1h to overnight; g) TFA, CH₂Cl₂,
 6 H₂O, r. t., overnight.

7
 8 We also chose 4-(3-benzamidophenyl)imidazole (nucleobase **D₃**) that can form two hydrogen bonds
 9 with T-A and C-G pairs and 4-(3-butylamidophenyl)imidazole (nucleobase **D₄**) that was found to
 10 recognize G-C base pairs with 3 hydrogen bonds (Figure S1 in Supporting Information) used for the
 11 previously prepared neomycin analogs. Furthermore, we decided to study two analogs of **D₃** and **D₄**. To
 12 this aim, we used the unsubstituted 3-aminophenylimidazole and the 4-(3-
 13 naphthylamidophenyl)imidazole in order to assess the influence of the substitution on the
 14 phenylimidazole moiety on biological activity. A new analog of the S-conjugate was also synthesized
 15 using a phenylthiazole moiety for comparison.²⁴ For the synthesis of these conjugates, we employed 1,3-
 16 dipolar cycloaddition leading to the formation of a triazole linker suited for direct comparison with the
 17 previously reported compounds but also to take advantage of a straightforward synthesis of these new
 18 analogs (Figure 1B).

19

20 **Synthesis of 2-DOS conjugates**

1 2-DOS is a meso compound that contains 5 contiguous stereogenic centers that pose a challenge for the
2 preparation of enantiomeric pure conjugates. To date, the most practical method to obtain 2-
3 deoxystreptomycin is by a straightforward acidic degradation of neomycin.²⁶ A more complex synthetic
4 route was previously reported for the preparation of enantiomerically pure compounds.³¹ In this work,
5 we first decided to employ the fastest synthetic pathway leading to desired conjugates as racemic
6 mixtures.

7 As illustrated in Scheme 1, 2-DOS moiety was prepared starting from commercially available neomycin
8 upon acidic hydrolysis in the presence of a concentrated solution of HCl. This led in quantitative yields
9 to neamine that was submitted to a further acidic treatment of aqueous HBr. The protection of the amino
10 groups with Boc protecting group was then performed on the crude product in the presence of tert-
11 butoxycarbonyl anhydride and NaOH in a mixture H₂O/dioxane 1:1 leading to compound **1** in 50% yield
12 over two steps. Next step was the protection of one vicinal diol in the presence of 1,1-
13 dimethoxycyclohexane and pTsOH in DMF that led to racemic compound (\pm)-**2** in 98% yield. Following
14 substitution of the remaining hydroxyl group with 2-azidoethyltosilate led to compound (\pm)-**3** ready for
15 1,3 dipolar cycloaddition in 33% yield. This latter was performed using appropriately modified
16 heteroaromatic compounds **10a-h** (Figure S2 in Supporting Information) containing the alkyne essential
17 for the coupling reaction. The cycloaddition reaction was performed in CH₃CN in the presence of CuI
18 and DIPEA and led to desired compounds (\pm)-**4a-h** in 46 to 87% yields. Final removal of the acetal and
19 Boc protecting groups in CH₂Cl₂ in the presence of H₂O and TFA led to desired compounds (\pm)-**5a-h** in
20 60 to 97% yields as TFA salts. Complete removal of copper traces possibly remaining in the final
21 product was performed upon treatment with Chelex resin. All synthesized compounds were also fully
22 characterized by NMR, HRMS and HPLC for purity and these data are shown in the Supporting
23 Information. The purity of the final products was included between 95% and 99%.

24

25 **Evaluation of the affinity, selectivity and inhibition activity**

26 Once the new series of 2-DOS conjugates **5a-h** prepared, we evaluated these compounds for their ability
27 to bind to pre-miR-372 and inhibit its processing by Dicer enzyme *in vitro* and compared the results to
28 the corresponding neomycin analogs when available. First of all, we studied the dissociation constants
29 (K_D) of all compounds for pre-miR-372 using a fluorescence-based assay where the targeted RNA is
30 labeled with a fluorophore (FAM) at the 5'-end. Binding of an efficient ligand induces a change in the
31 fluorophore environment that quenches fluorescence and the variation of the fluorescence signal in
32 function of the concentration of ligand allows for the measurement of K_D .³² As illustrated in Table 1, all
33 newly synthesized compounds **5a-h** are good pre-miR-372 ligands with K_D in the low μ M range (from
34 1.62 to 17.1 μ M) except for **5c** that bears a higher K_D of 101 μ M. It should be noted that 2-DOS and
35 nucleobases alone have no affinity for the target. A closer look at the obtained K_D suggests that **D₃** and
36 **D₄** analogs (**5d** and **5e**, respectively) are the strongest binders closely followed by the unsubstituted
37 phenylimidazole analog **5g**. Introduction of a naphthalene substituent as in **5f** and of the phenylthiazole

1 substituent as in **5h** induces a slight loss of affinity while the natural nucleobases conjugates **5a-c** are
 2 the weakest binders.

3

4 **Table 1.** Dissociation constants values in μM for synthesized compounds **5a-h** toward pre-miR-372
 5 alone (K_D) and in the presence of tRNA (100 eq., K_D') or DNA (100 eq. K_D'').

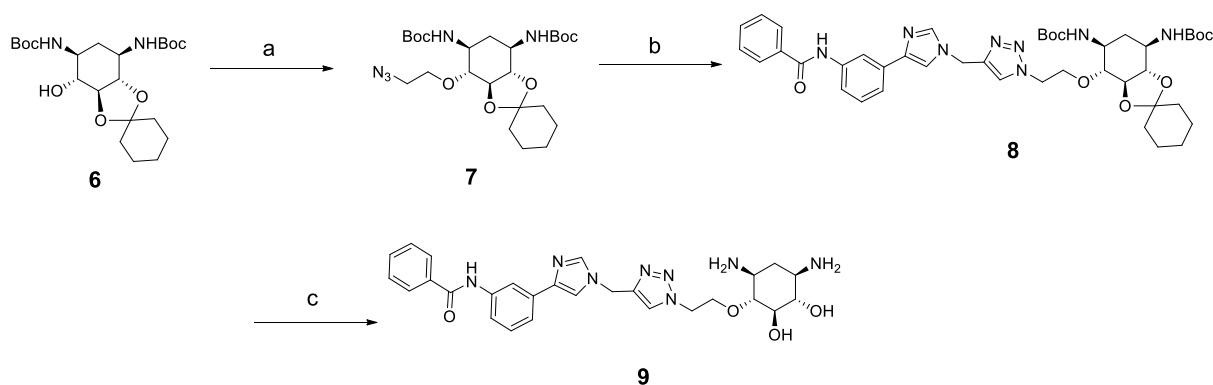
ID	K_D	K_D'	K_D'/K_D	K_D''	K_D''/K_D	IC_{50}	IC_{50}'
5a	11.1 ± 2.3	> 1 mM	-	47.1	4.2	no inhibition	no inhibition
5b	17.1 ± 0.1	> 1 mM	-	> 1 mM	-	no inhibition	no inhibition
5c	101 ± 18	> 1 mM	-	> 1 mM	-	no inhibition	no inhibition
5d	2.52 ± 0.58	2.64 ± 0.52	1.0	2.96 ± 0.85	1.2	15.9 ± 1.8	29.5 ± 9.1
5e	1.62 ± 0.49	3.65 ± 0.85	2.2	2.11 ± 0.81	1.3	72.4 ± 1.0	no inhibition
5f	6.78 ± 0.46	8.51 ± 1.3	1.3	9.02 ± 1.2	1.3	> 100 μM	no inhibition
5g	3.53 ± 0.89	3.92 ± 0.52	1.1	4.27 ± 0.35	1.2	81.1 ± 2.3	> 500 μM
5h	5.13 ± 0.41	8.56 ± 2.0	1.7	11.0 ± 0.90	2.1	74.9 ± 12	no inhibition
Neo-D₃	0.0189 ± 0.0017	0.0208 ± 0.0019	1.1	0.0244 ± 0.0018	1.3	8.30 ± 1.1	20.4
Neo-D₄	0.0461 ± 0.0038	0.0164 ± 0.0012	1.1	0.0222 ± 0.0024	1.5	25.6 ± 1.1	
2-DOS	> 1 mM	-	-	-	-	no inhibition	no inhibition

6

7 Selectivity is of course another important parameter to evaluate. To this aim, we chose to measure K_D
 8 in the presence of two different competitors, tRNA and DNA, that are particularly abundant in cells. In
 9 both cases, 100 equivalents of competitor were added to the mixture during K_D measurement. An
 10 increase in K_D (ratio K_D'/K_D for tRNA or K_D''/K_D for DNA higher than one) suggests a lack of selectivity
 11 since the compound can bind to other nucleic acid structures. The obtained results are illustrated in Table
 12 1 and suggest that natural nucleobase conjugates **5a-c** were not selective in the presence of tRNA and
 13 DNA except for **5a** that maintained some affinity in the presence of DNA ($K_D''/K_D = 4.2$). Compounds
 14 **5d-5h** maintained their affinity and seemed thus to be selective for a pre-miRNA structure such as pre-
 15 miR-372, compared to other nucleic acid structures.

16 With these experiments we assessed that all compounds were promising binders and that compounds
 17 **5d-5h** were also selective for pre-miRNAs. We thus decided to verify if the compounds were also able
 18 to inhibit Dicer processing of pre-miR-372 by measuring the IC_{50} using a previously published cell-free
 19 assay where the pre-miR-372 was double labeled with a fluorophore (FAM) and a quencher (DAB) at
 20 the 5'- and at the 3'-end, respectively.²³ When Dicer cleaves the pre-miRNA appropriately the
 21 fluorescence signal increases, while when Dicer cleavage is inhibited no fluorescence is observed. As
 22 shown by IC_{50} values reported in Table 1, compounds **5a-5c** were not able to induce inhibition and
 23 compounds **5e-5h** had a weak inhibition activity starting at 72.4 μM for **5e** to more than 100 μM for **5f**.
 24 Noteworthy, **5d** was the most active compound with an IC_{50} of 15.9 μM . This value is in the same range
 25 of the one of compound **Neo-D₃** while this latter bears a much higher affinity for the target. This could

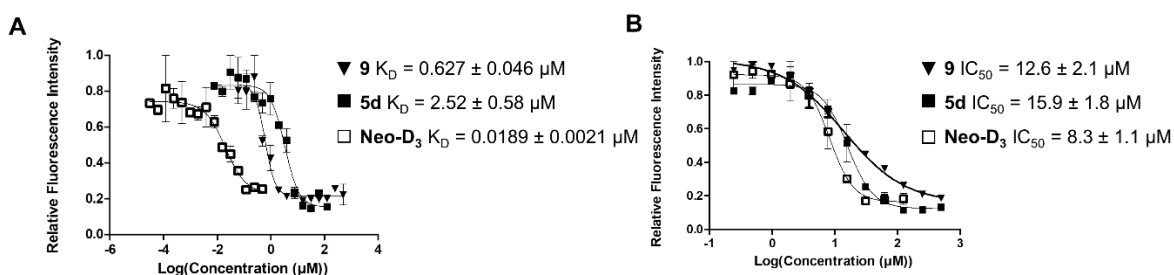
1 be explained by a difference in the binding site that could be more favorable for inhibition in the case
 2 of compound **5d** than of compound **Neo-D₃** despite a loss in binding affinity.
 3 Selectivity remains a major concern when designing new RNA ligands. We thus evaluated IC₅₀s in the
 4 presence of HEK293 cells lysates instead of using the recombinant enzyme. These conditions mimic the
 5 intracellular complexity and give hints about the potential selectivity in cells. With these experiments
 6 we demonstrated that all compounds lost their ability to inhibit miR-372 maturation except for
 7 compound **5d** whose IC₅₀' is 29.5 μM instead of 15.9 μM in the presence of pure enzyme.
 8 Despite the binding selectivity for all compounds against tRNA and DNA as well as the inhibition
 9 selectivity in the case of compound **5d**, one could wonder what would be the affinity and the inhibition
 10 activity of these compounds against other pre-miRNAs. We thus measured K_D and IC₅₀ of the newly
 11 synthesized compounds for three supplementary pre-miRNAs: pre-miR-21, pre-miR-18a and pre-miR-
 12 148a. These three miRNAs are oncogenic and overexpressed in many types of cancers.³³ Although their
 13 sequences and structures differ from those of pre-miR-372, the general shape of pre-miRs remains
 14 similar, i.e. hairpin stem-loops with bulges. The obtained results (Table S1 in the Supporting
 15 Information) show that compounds **5a-c**, the weakest binders of pre-miR-372, remain weak binders or
 16 loose affinity for the other miRNAs. Furthermore, **5a-c** did not show any ability to inhibit Dicer
 17 cleavage. Compounds **5d-h** instead maintain similar affinity for all pre-miRNAs, but show major
 18 differences in inhibition activity. Indeed, compound **5d** inhibits weakly pre-miR-21 maturation and is
 19 not active on pre-miR-18. Inhibition activity is maintained for pre-miR-148a but is lower than the one
 20 for pre-miR-372. The weak inhibitors of pre-miR-372 maturation **5e-h** remain weak for the other tested
 21 miRNAs except for **5e** that inhibits preferentially pre-miR-18 cleavage (IC₅₀ 20.3 μM) and pre-miR-
 22 148a (IC₅₀ 18.2 μM). Compound **5d** thus seems to be the most promising of the series for the inhibition
 23 of pre-miR-372 processing with IC₅₀ in the low μM range similar to one of its neomycin counterpart
 24 and yet having a much higher K_D value.
 25 As mentioned above, compounds **5a-h** are racemic mixture. We thus wanted to verify if the enantiopure
 26 analog of the most promising compound of the series **5d** showed any difference in activity, affinity and
 27 selectivity. The synthesis of this compound is similar to the one of **5d** but starts from the enantiopure
 28 compound **6** prepared following a previously reported procedure (Scheme 2).³¹



1 **Scheme 2.** Synthesis of enantiopure compound **9**. Reagents: a) 2-Azidoethyl-p-toluensulfonate, NaH,
2 THF, 30°C, 3h; b) **10h**, CuI, DIPEA, CH₃CN, r. t., 5h; c) TFA, CH₂Cl₂, H₂O, r. t., overnight.

3
4 The introduction of the 2-azidoethyl linker led to compound **7** in 20% yield and the 1,3-dipolar
5 cycloaddition in the presence of 4-(3-benzamidophenyl)imidazole led to compound **8** in 56% yield. Final
6 deprotection in acidic conditions allowed desired derivative **9** in 98% yield. The comparison of the
7 affinity for pre-miR-372 between **9** and **5d** revealed that compound **9** was a stronger binder with a K_D
8 of 0.627 μM. This improved affinity was then reflected in a better inhibition activity since **9** showed a
9 IC₅₀ of 12.6 μM. This enantiomer thus seems more favorable for binding and inhibition.

10 Altogether these results show that there is a good correlation between K_D and IC₅₀ suggesting that RNA
11 binding is the underlying mechanism of inhibition. Furthermore, the result obtained with compound **9**
12 is particularly interesting since comparison of IC₅₀ between **9** and compound **Neo-D₃** shows that these
13 are within the same range (8.3 μM vs 12.6 μM, Figure 2). Neomycin could thus be replaced by 2-DOS
14 without losing activity.



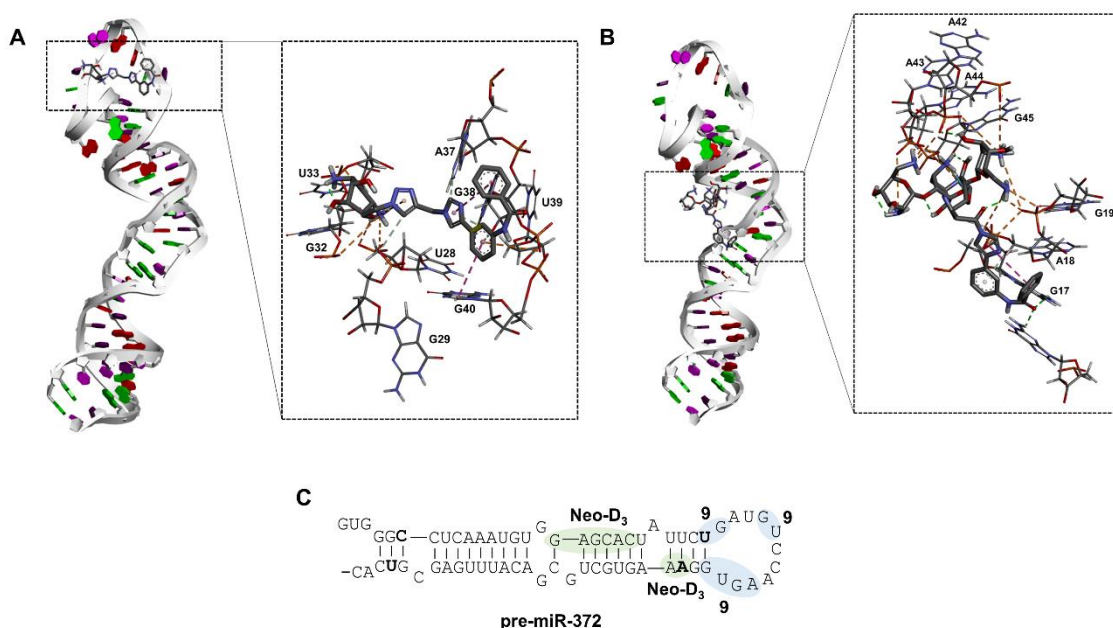
15
16 **Figure 2.** Comparison of the dissociation constants (K_D, A) and of inhibition activities, (IC₅₀, B) for
17 compounds **9**, **5d** and **Neo-D₃** against pre-miR-372.

18
19 The explanation for the good inhibition activity of compound **9** despite a higher K_D value could come
20 from a more favorable binding site for pre-miR-372 compared to the one of **Neo-D₃**. This parameter
21 could also explain the differences observed in the inhibition activities of other pre-miRNAs while
22 keeping the affinity for them. Indeed, binding to a particular RNA is not sufficient to induce an inhibition
23 activity since it is essential to bind to a functional site that in this case would be preferentially the
24 cleavage site of Dicer enzyme.

25 26 **Molecular docking of compound **9** on pre-miR-372**

27 To gain a better insight into the mode of binding of **9** and suggest an explanation for the good inhibition
28 activity of this compound similar to the one of the **Neo-D₃** analog despite a much higher K_D, we
29 performed a molecular docking study of both compounds on the pre-miR-372 sequence. The sequence
30 of the pre-miR-372 hairpin loop was generated from the miRBase database and then input to the MC-
31 Fold/MC-Sym pipeline to construct a 3D model. For this model, we analyzed energy optimization using

1 TINKER. Next, we conducted molecular docking for pre-miR-372 in the presence of compound **9** using
 2 the AutoDock program without defining any preferred binding site but performing the docking study on
 3 the entire sequence.³⁴ As shown in Figure 3, docking suggested that both compounds **9** and **Neo-D₃** bind
 4 to the cleavage site of Dicer even if differently. Compound **Neo-D₃** seems to interact with residues A45
 5 and A46 corresponding to the cleavage site on one strand of pre-miR-372 stem, while **9** with U31 and
 6 G32 corresponding to the cleavage site on the opposite strand. Regarding the other interactions, **Neo-D₃**
 7 interacts with other residues of the stem region (G20 to C25) while **9** with residues of the loop region
 8 (G35-U36 and A40-G43). Both these binding sites involving the cleavage site of the enzyme, the
 9 compounds behave similarly in terms of inhibition activity.



10
 11 **Figure 3.** Molecular docking of compound DOS-D₃ **9** (A) and **Neo-D₃** (B) performed on pre-miR-372
 12 structure using Autodock 4 software. Primary and secondary structure of pre-miR-372 (C) and binding
 13 sites of compound **9** (light blue) and **Neo-D₃** (light green).

14
 15 In conclusion, we were able to synthesize a new series of 2-desoxystreptamine analogs conjugated to
 16 various heteroaromatic compounds such as natural and artificial nucleobases as new RNA binders. We
 17 performed a comparison of affinity, selectivity, inhibition activity and site of interaction for oncogenic
 18 pre-miR-372 with a previously synthesized series of ligands containing the neomycin aminoglycoside
 19 instead of 2-DOS. The aim of this work was to reduce the overall size and hydrophilicity of the RNA
 20 ligands and to verify if the biological properties could be maintained. We observed that all synthesized
 21 compounds are good RNA binders with selectivity when tested in competition with other nucleic acid
 22 structures. Some compounds were able to inhibit miR-372 maturation upon inhibiting Dicer cleavage of
 23 pre-miR-372 but only compound **5d** showed an IC₅₀ in the low micromolar range. Some selectivity was
 24 also observed once the compound tested against other pre-miRNAs. However, we already observed that

1 compounds that are selective for a small set of pre-miRNAs can have a very specific biological effect
2 and this could be an advantage for a comprehensive biological activity. The same evaluations performed
3 on the enantiopure analog of **5d**, compound **9**, showed that this latter was an even better ligand and
4 inhibitor concerning pre-miR-372. Finally, it was observed that both **5d** and **9** are weaker binders of pre-
5 miR-372 compared to the neomycin counterpart **Neo-D₃**, but that they maintained a similar inhibition
6 activity. Molecular docking suggested that this is due to the particular binding site that is located on the
7 nucleotides that are cleaved by Dicer enzyme thus being functional for efficient inhibition. Further
8 chemical optimization of these 2-DOS conjugates could thus lead to even better inhibition activity and
9 to study their intracellular activity.

10

11 **Acknowledgments**

12 We thank Dr. J. M. Guignon (Plateforme Bernard Rossi, CEA TIRO, Nice, France) for HRMS analyses
13 and Agence Nationale de la Recherche (ANR, France; ANR-11-JS07-011-01) that funded this work.
14 PhD fellowship to T.P.A.T. was supported by the Vietnam Ministry of Science and Technology (VIED
15 grant 911), to S.P. by Université Côte d'Azur IDEX-JEDI, to M.P. by ANRT and Sanofi. A post-doc
16 fellowship to A.R. was supported by Université Côte d'Azur.

17

1 **Figures and Schemes**

2 **Figure 1.** General structure of previously synthesized neomycin-nucleobase conjugates (A) and of the
3 newly designed 2-DOS-nucleobase conjugates (B). The detailed structure for nucleobases **S**, **D₃** and **D₄**
4 is illustrated in the dashed square.

5 **Figure 2.** Comparison of the dissociation constants (K_D , A) and of inhibition activities, (IC_{50} , B) for
6 compounds **9**, **5d** and **Neo-D₃** against pre-miR-372.

7 **Figure 3.** Molecular docking of compound DOS-D₃ **9** (A) and **Neo-D₃** (B) performed on pre-miR-372
8 structure using Autodock 4 software. Primary and secondary structure of pre-miR-372 (C) and binding
9 sites of compound **9** (light blue) and **Neo-D₃** (light green).

10 **Scheme 1.** Synthesis of the new series of 2-DOS conjugates **5a-h**. Reagents: a) HCl, reflux, overnight;
11 b) 48% HBr aq., reflux, 2 days; c) Boc₂O, NaOH, dioxane/H₂O, overnight; d) 1,1-
12 dimethoxycyclohexane, pTsOH, DMF, 50°C, 55 mbar, 1h; e) 2-Azidoethyl-p-toluensulfonate, NaH,
13 THF, 30°C, 3h; f) **10a-h**, CuI, DIPEA, CH₃CN, from r. t. to 50°C, from 1h to overnight; g) TFA, CH₂Cl₂,
14 H₂O, r. t., overnight.

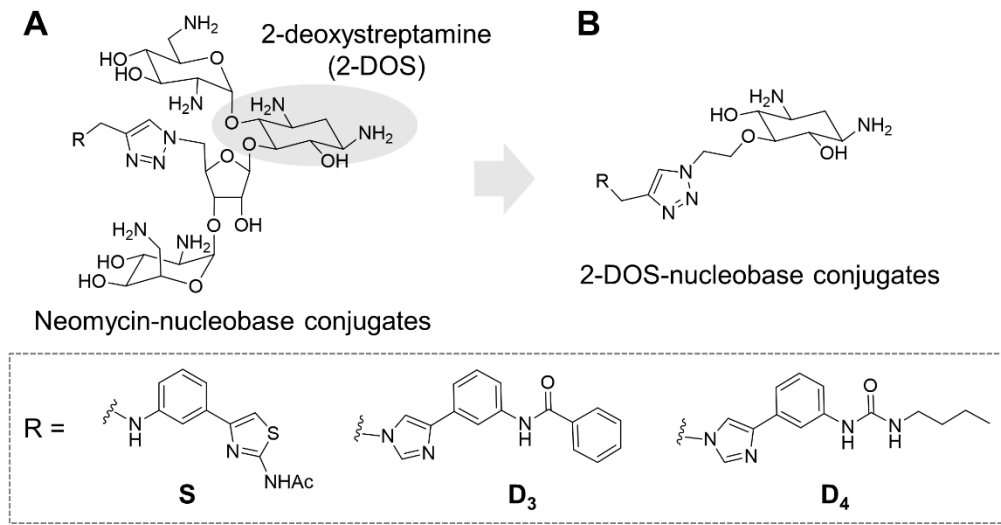
15 **Scheme 2.** Synthesis of enantiopure compound **9**. Reagents: a) 2-Azidoethyl-p-toluensulfonate, NaH,
16 THF, 30°C, 3h; b) **10h**, CuI, DIPEA, CH₃CN, r. t., 5h; c) TFA, CH₂Cl₂, H₂O, r. t., overnight.

17

18

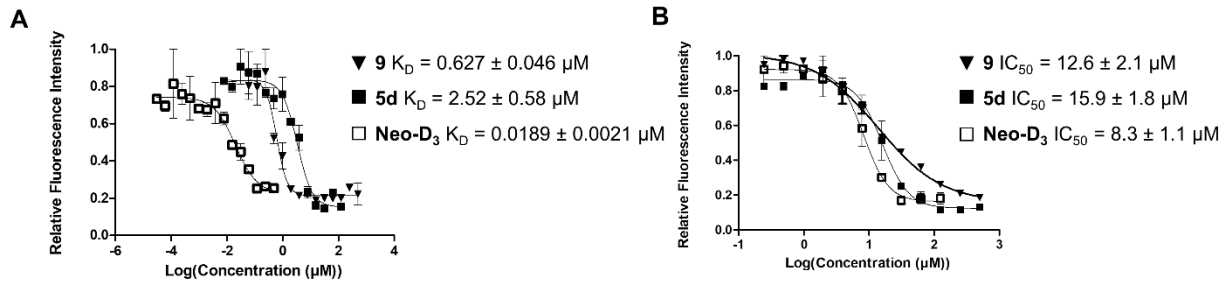
19

1 **Figure 1.**



2
3
4

1 **Figure 2.**

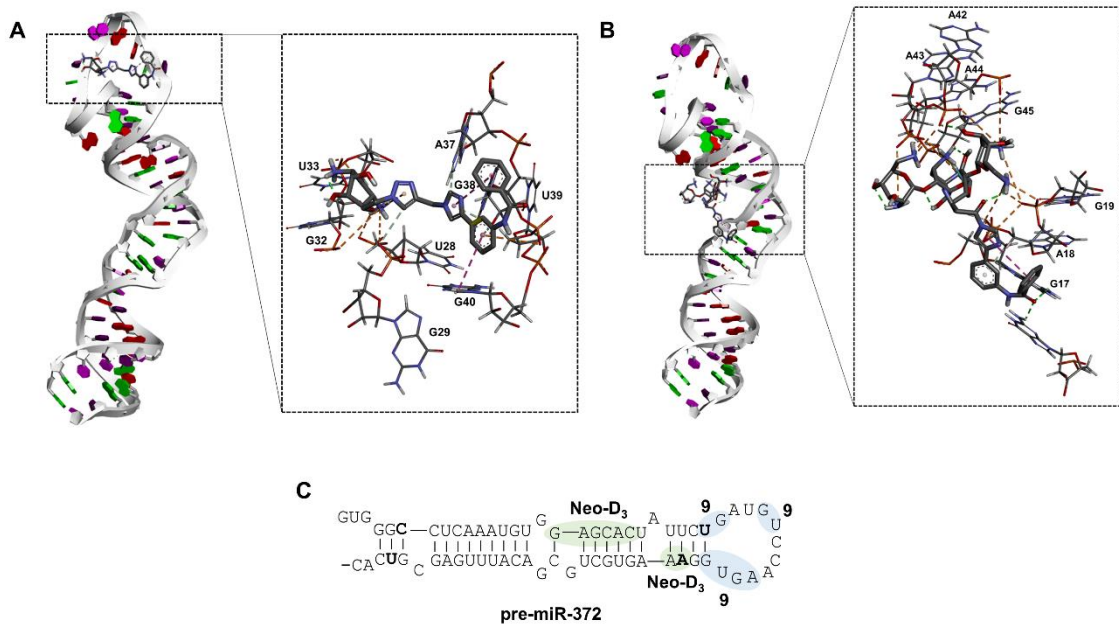


2

3

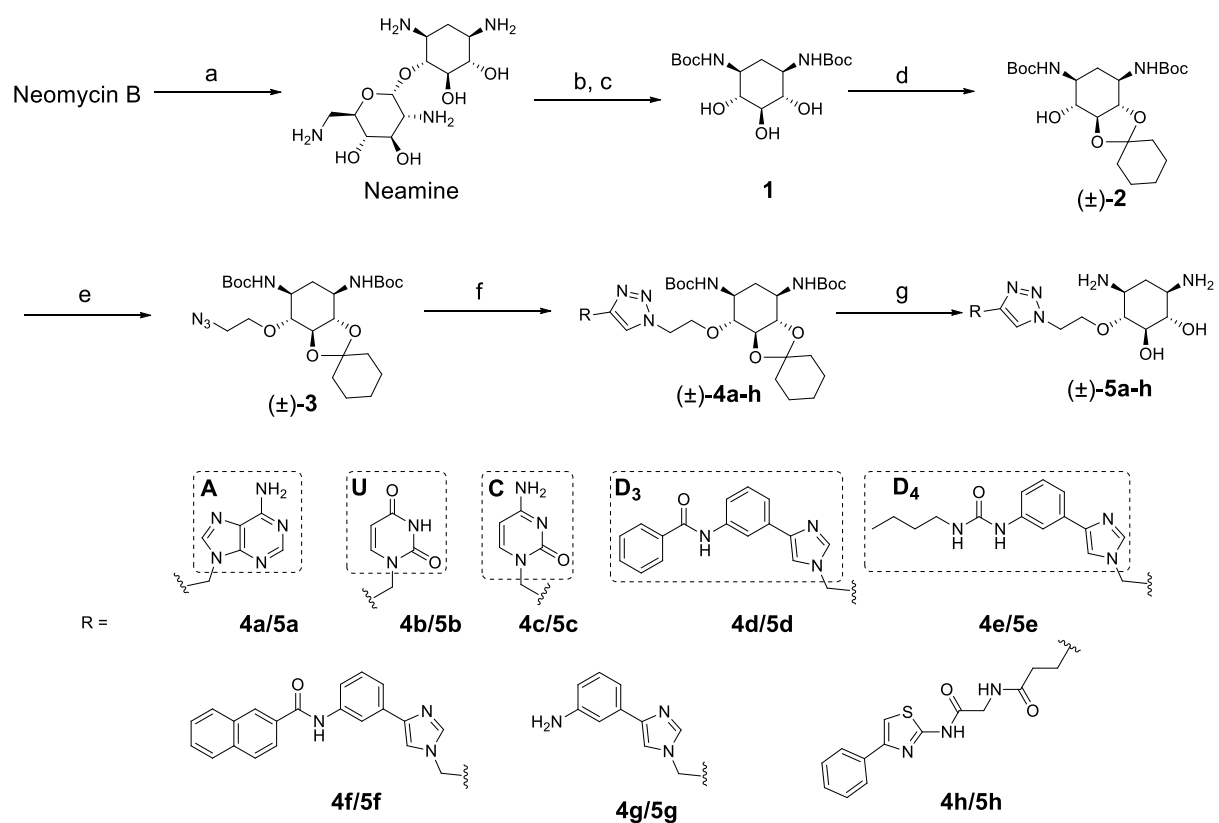
4

1 **Figure 3.**



- 2
- 3
- 4

1 **Scheme 1.**

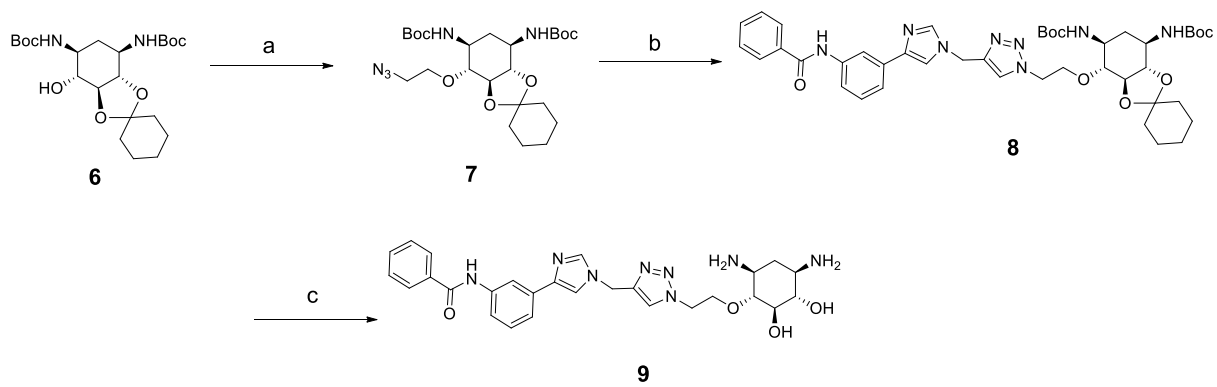


2

3

4

1 **Scheme 2.**



2

3

- 1 **Table 1.** Dissociation constants values in μM for synthesized compounds **5a-h** toward pre-miR-372
 2 alone (K_D) and in the presence of tRNA (100 eq., K_D') or DNA (100 eq. K_D'').

ID	K_D	K_D'	K_D'/K_D	K_D''	K_D''/K_D	IC_{50}	IC_{50}'
5a	11.1 ± 2.3	> 1 mM	-	47.1	4.2	no inhibition	no inhibition
5b	17.1 ± 0.1	> 1 mM	-	> 1 mM	-	no inhibition	no inhibition
5c	101 ± 18	> 1 mM	-	> 1 mM	-	no inhibition	no inhibition
5d	2.52 ± 0.58	2.64 ± 0.52	1.0	2.96 ± 0.85	1.2	15.9 ± 1.8	29.5 ± 9.1
5e	1.62 ± 0.49	3.65 ± 0.85	2.2	2.11 ± 0.81	1.3	72.4 ± 1.0	no inhibition
5f	6.78 ± 0.46	8.51 ± 1.3	1.3	9.02 ± 1.2	1.3	> 100 μM	no inhibition
5g	3.53 ± 0.89	3.92 ± 0.52	1.1	4.27 ± 0.35	1.2	81.1 ± 2.3	> 500 μM
5h	5.13 ± 0.41	8.56 ± 2.0	1.7	11.0 ± 0.90	2.1	74.9 ± 12	no inhibition
Neo-D₃	0.0189 ± 0.0017	0.0208 ± 0.0019	1.1	0.0244 ± 0.0018	1.3	8.30 ± 1.1	20.4
Neo-D₄	0.0461 ± 0.0038	0.0164 ± 0.0012	1.1	0.0222 ± 0.0024	1.5	25.6 ± 1.1	
2-DOS	> 1 mM	-	-	-	-	no inhibition	no inhibition

3

4

1 References

- 2 1. J. P. Falese, A. Donlic and A. E. Hargrove, *Chem Soc Rev*, 2021, **50**, 2224-2243.
- 3 2. K. D. Warner, C. E. Hajdin and K. M. Weeks, *Nat Rev Drug Discov*, 2018, **17**, 547-558.
- 4 3. D. N. Wilson, *Nat Rev Microbiol*, 2014, **12**, 35-48.
- 5 4. H. Ratni, R. S. Scalco and A. H. Stephan, *ACS Med Chem Lett*, 2021, **12**, 874-877.
- 6 5. A. Di Giorgio and M. Duca, *MedChemComm*, 2019, DOI: 10.1039/C1039MD00195F.
- 7 6. V. Ambros, *Nat Med*, 2008, **14**, 1036-1040.
- 8 7. M. Winkle, S. M. El-Daly, M. Fabbri and G. A. Calin, *Nat Rev Drug Discov*, 2021, **20**, 629-651.
- 9 8. F. Zamani and T. Suzuki, *J Med Chem*, 2021, **64**, 7110-7155.
- 10 9. Z. Li and T. M. Rana, *Nat Rev Drug Discov*, 2014, **13**, 622-638.
- 11 10. S. T. Crooke, B. F. Baker, R. M. Crooke and X. H. Liang, *Nat Rev Drug Discov*, 2021, **20**, 427-
12 453.
- 13 11. A. Di Giorgio, T. P. Tran and M. Duca, *Future Med Chem*, 2016, **8**, 803-816.
- 14 12. M. D. Disney and A. J. Angelbello, *Acc Chem Res*, 2016, **49**, 2698-2704.
- 15 13. M. D. Disney, A. M. Winkelsas, S. P. Velagapudi, M. Southern, M. Fallahi and J. L. Childs-
16 Disney, *ACS Chem Biol*, 2016, **11**, 1720-1728.
- 17 14. S. P. Velagapudi, M. D. Cameron, C. L. Haga, L. H. Rosenberg, M. Lafitte, D. R. Duckett, D. G.
18 Phinney and M. D. Disney, *Proc Natl Acad Sci U S A*, 2016, **113**, 5898-5903.
- 19 15. S. P. Velagapudi, S. M. Gallo and M. D. Disney, *Nat Chem Biol*, 2014, **10**, 291-297.
- 20 16. F. A. Abulwerdi, W. Xu, A. A. Ageeli, M. J. Yonkunas, G. Arun, H. Nam, J. S. Schneekloth, Jr., T.
21 K. Dayie, D. Spector, N. Baird and S. F. J. Le Grice, *ACS Chem Biol*, 2019, **14**, 223-235.
- 22 17. K. Gumireddy, D. D. Young, X. Xiong, J. B. Hogenesch, Q. Huang and A. Deiters, *Angew Chem*
23 *Int Ed Engl*, 2008, **47**, 7482-7484.
- 24 18. Z. Shi, J. Zhang, X. Qian, L. Han, K. Zhang, L. Chen, J. Liu, Y. Ren, M. Yang, A. Zhang, P. Pu and
25 C. Kang, *Cancer Res*, 2013, **73**, 5519-5531.
- 26 19. C. Staedel, T. P. A. Tran, J. Giraud, F. Darfeuille, A. Di Giorgio, N. J. Tourasse, F. Salin, P. Uriac
27 and M. Duca, *Sci Rep*, 2018, **8**, 1667.
- 28 20. J. Zhang, S. Umemoto and K. Nakatani, *J Am Chem Soc*, 2010, **132**, 3660-3661.
- 29 21. C. Maucort, D. D. Vo, S. Aouad, C. Charrat, S. Azoulay, A. Di Giorgio and M. Duca, *ACS Med*
30 *Chem Lett*, 2021, **12**, 899-906.
- 31 22. D. D. Vo, C. Becquart, T. P. A. Tran, A. Di Giorgio, F. Darfeuille, C. Staedel and M. Duca, *Org*
32 *Biomol Chem*, 2018, **16**, 6262-6274.
- 33 23. D. D. Vo, C. Staedel, L. Zehnacker, R. Benhida, F. Darfeuille and M. Duca, *ACS Chem Biol*,
34 2014, **9**, 711-721.
- 35 24. D. D. Vo, T. P. Tran, C. Staedel, R. Benhida, F. Darfeuille, A. Di Giorgio and M. Duca, *Chemistry*,
36 2016, **22**, 5350-5362.
- 37 25. V. Malnuit, M. Duca and R. Benhida, *Org Biomol Chem*, 2011, **9**, 326-336.
- 38 26. S. Magnet and J. S. Blanchard, *Chem Rev*, 2005, **105**, 477-498.
- 39 27. S. Yoshizawa, D. Fourmy, R. G. Eason and J. D. Puglisi, *Biochemistry*, 2002, **41**, 6263-6270.
- 40 28. C. M. Klemm, A. Berthelmann, S. Neubacher and C. Arenz, *Eur J Org Chem*, 2009, 2788-2794.
- 41 29. X. Liu, J. R. Thomas and P. J. Hergenrother, *J Am Chem Soc*, 2004, **126**, 9196-9197.
- 42 30. J. R. Thomas, X. Liu and P. J. Hergenrother, *J Am Chem Soc*, 2005, **127**, 12434-12435.
- 43 31. C. M. Klemm, A. Berthelmann, S. Neubacher and C. Arenz, *European Journal of Organic*
44 *Chemistry*, 2009, **2009**, 2788-2794.
- 45 32. B. Llano-Sotelo, E. F. Azucena, Jr., L. P. Kotra, S. Mobashery and C. S. Chow, *Chem Biol*, 2002,
46 **9**, 455-463.
- 47 33. T. Dalmay and D. R. Edwards, *Oncogene*, 2006, **25**, 6170-6175.
- 48 34. G. M. Morris, R. Huey, W. Lindstrom, M. F. Sanner, R. K. Belew, D. S. Goodsell and A. J. Olson,
49 *J Comput Chem*, 2009, **30**, 2785-2791.

50