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Novel 2,5-Dideoxystreptamine Derivatives Targeting the Ribosomal Decoding Site RNA

Dionisios Vourloumis,[a,*] Masayuki Takahashi,[a] Geoffrey C. Winters,[a] Klaus B. Simonsen,[a] Benjamin K. Ayida,[a] Sofia Barluenga,[a] Seema Qamar,[b] Sarah Shandrick,[b] Qiang Zhao[b] and Thomas Hermann[b,*]

[a]*Department of Medicinal Chemistry, Anadys Pharmaceuticals, Inc., 9050 Camino Santa Fe, San Diego, CA 92121, USA*
[b]*Departments of RNA Biochemistry and Computational Chemistry and Structure, Anadys Pharmaceuticals, Inc., 9050 Camino Santa Fe, San Diego, CA 92121, USA*

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Abstract—The ribosomal decoding site is the target of aminoglycoside antibiotics that specifically recognize an internal loop RNA structure. We synthesized RNA-targeted 2,5-dideoxystreptamine-4-amides in which a sugar moiety in natural aminoglycosides is replaced by heterocycles.

The bacterial ribosome is the primary target for a variety of antibiotics that have been shown to interact predominantly with the ribosomal RNA (rRNA) components.[1,2] The decoding site internal loop RNA (Fig. 1B) within the 30S ribosomal subunit is specifically recognized by aminoglycoside antibiotics[3] such as neomycin (**1**) and paromomycin (**2**) (Fig. 1A) that, upon binding, interfere with translation fidelity,[4] which ultimately leads to bacterial cell death. The molecular basis of decoding site RNA recognition by natural aminoglycosides[1,2,5–8] and synthetic derivatives[9–12] has been subject to numerous studies. For both the decoding site RNA and the whole 30S ribosomal subunit complexed with aminoglycosides three-dimensional structures have been determined recently,[2,6,7] providing the foundation for structure-based design of novel antibiotics[13] superior to aminoglycosides. The efficacy of the natural antibiotics is compromised by bacterial resistance[14] and undesirable pharmacological profiles. Nevertheless, aminoglycosides are recognized as the lead paradigm in RNA target recognition,[15] providing starting points for the design of RNA binders. The immense challenge of selective derivatization of the natural aminoglycosides, however, has led the effort to use smaller fragments, such as neamine (**3**),[10,12,16] paromamine (**4**),[11,17] and 2-deoxystreptamine (2-DOS) (**5**)[18–20] (Fig. 1A), as scaffolds for synthesis. The 2-DOS core, conserved among biologically active aminoglycosides, lends itself to the construction of decoding site-targeted ligands, as it contains a unique spatial arrangement of amino groups, that is deemed crucial to the recognition of the bacterial rRNA target.[2,6,7] Moreover, 2-DOS is readily available by chemical degradation of natural aminoglycosides.[21]

Herein, we describe the design, synthesis, and preliminary testing of 2-DOS-4-carboxyl amides. Structural data from X-ray crystallography,[2,7] along with our own molecular modelling studies[22] and a previous investigation,[19] suggested that the pyranose ring, linked to the 4-position of 2-DOS in many natural aminoglycosides, might be replaced by planar non-sugar moieties that combine the potential for both stacking and hydrogen bonding interactions, along with straightforward accessibility by medicinal chemistry. In a similar previous study, the 4-hydroxyl group of 2-DOS served as a handle for the formation of a variety of ethers, yielding weak binding nonglycosidic 2-DOS derivatives that displayed affinity for the bacterial decoding site RNA in the range of 300 μM and higher.[19] Our design is based on amides formed through an activated carboxyl group introduced at the 4-position of 2-DOS. The amide

*Corresponding author. Tel.: +1-858-527-3648/3659; fax: +1-858-527-1539; e-mail: dvourloumis@anadyspharma.com (D. Vourloumis); thermann@anadyspharma.com (T. Hermann).

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functionality provides a rigid linkage between the attached scaffold and 2-DOS, and may allow for additional hydrogen bond interactions with the RNA target. We hypothesized that both the increased compound rigidity and hydrogen bonding potential might yield nonglycosidic 2-DOS derivatives of improved affinity for the decoding site RNA.

The starting material for our synthetic efforts was obtained by hydrolytic degradation of commercially available neomycin sulfate (**1**), as previously described (Scheme 1).[21] Thus, treatment of **1** with concentrated HBr (48%) under reflux conditions produced the dihydrobromide salt of 2-DOS (**5**) in 91% yield. The amine-functionalities of 2-DOS were protected as the corresponding azides using triflic azide[23] in the presence of a catalytic amount of $CuSO_4$.[24] Subsequent treatment with acetic anhydride and 4-DMAP (catalytic) in pyridine furnished the peracetylated product in 87% yield over two steps. Enzymatic resolution by Novozym 435 in toluene/potassium phosphate buffer (pH 6.2) produced optically pure **6** in 96% yield.[10]

Various different protecting strategies were examined at this point for their compatibility with the planned synthetic sequence. Specifically, silicon-protecting groups proved to be highly prone to migration under the basic reaction conditions used in the following steps (Scheme 1). Similar results were obtained from the use of esters for the protection of the hydroxyl-groups, that led to loss of optical purity due to migration between positions 4, 5, and 6. Methoxymethyl-ethers were unsuitable due to their unique deprotection requirements (strong protic or Lewis acids) interfering with other functionalities at later stages of the synthesis. Benzylic ethers proved to be compatible with the desired chemistry, especially

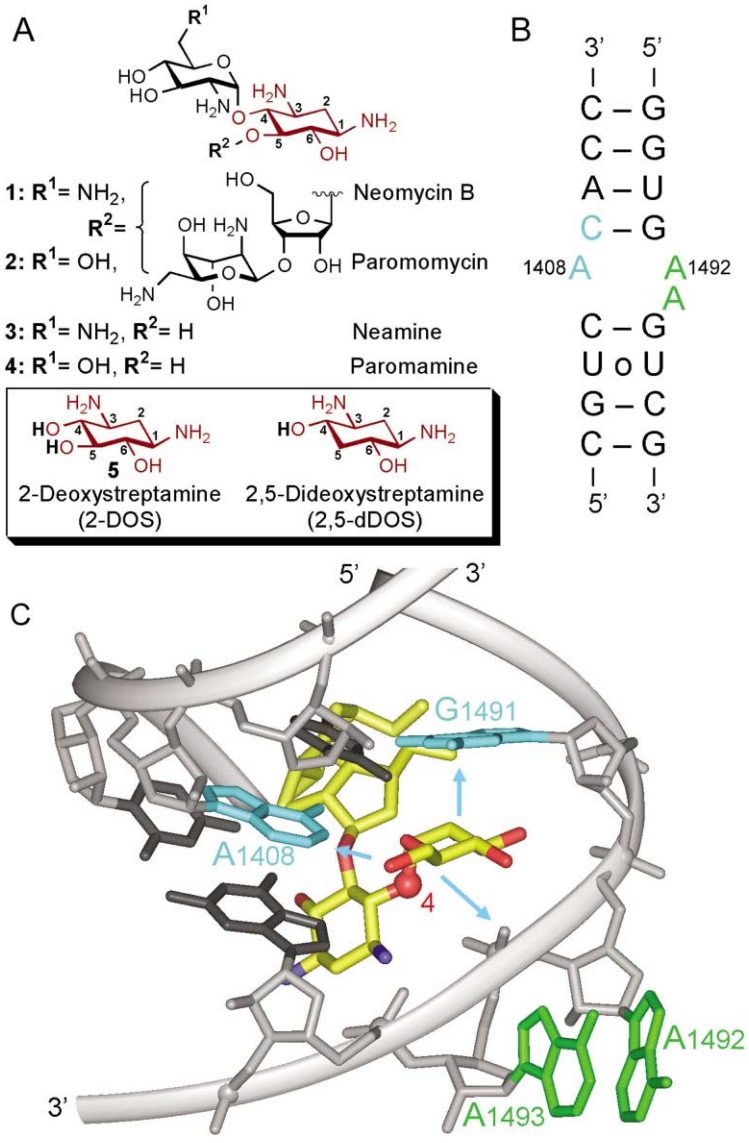
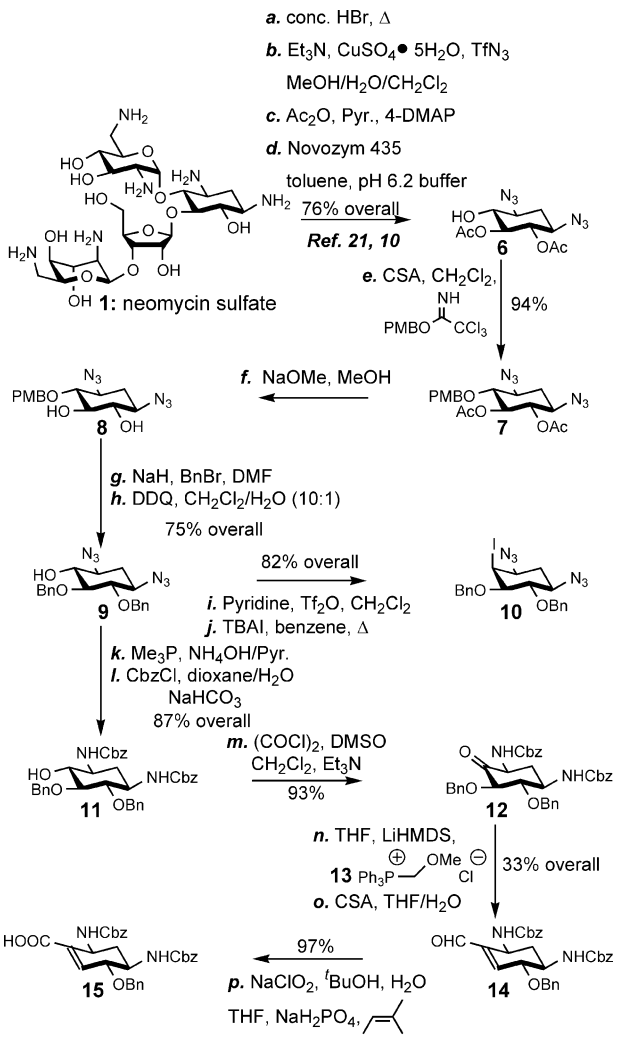


Figure 1. (A) 2-Deoxystreptamine (2-DOS) (**5**), the conserved core scaffold of naturally occurring aminoglycoside antibiotics such as neomycin (**1**), paromomycin (**2**), neamine (**3**) and paromamine (**4**) that bind specifically to the decoding site of bacterial rRNA. (B) Secondary structure of the bacterial decoding site rRNA. (C) Three-dimensional structure of paromomycin (yellow) bound to the bacterial decoding site rRNA.[2] The flipped-out adenine residues A1492 and A1493 are in green color. The bases A1408 and G1491, which interact with the 2-DOS ring by, respectively, hydrogen bonding and stacking are colored cyan.

because of their introduction under mildly acidic conditions, as well as their stability to base treatment. Specifically, reaction of (**6**) with *p*-methoxybenzyl trichloroacetimidate [PMBOC(=NH)CCl_3] in the presence of a catalytic amount of CSA,[25] furnished the desired PMB-ether **7** in 94% yield. Hydrolysis of the two acetates in **7** with NaOMe in methanol, followed by di-benzylation (BnBr, NaH) and oxidative removal of the PMB-functionality, produced advanced intermediate **9** in 75% overall yield.



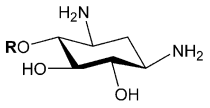
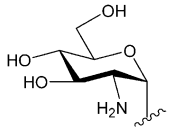
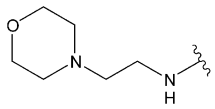
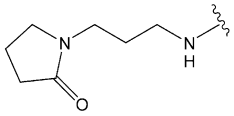
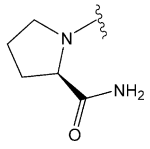
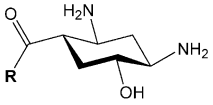
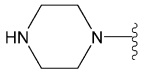
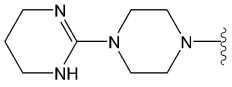
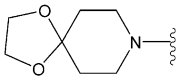
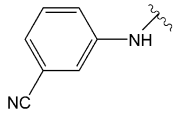
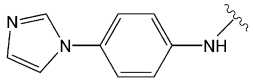
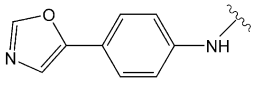
Scheme 1. Reagents and conditions: (a) 1.0 equiv of neomycin sulfate, 48% HBr (0.2 M), 20 h, reflux, 91%; (b) 1.0 equiv of 2-deoxystreptamine·2HBr, 3.0 equiv of TfN_3, 0.1 equiv of $CuSO_4{\cdot}5H_2O$, 10.0 equiv of Et_3N, MeOH (0.1 M), 16 h, 23 °C; (c) 1.0 equiv of diazidotriol, 24.0 equiv of pyridine, 10.0 equiv of Ac_2O, 0.1 equiv of 4-DMAP, 16 h, 0→23 °C, 87% over two steps; (d) 1.0 equiv of triacetate, Novozym 435 (1:1 w/w), toluene/potassium phosphate buffer (pH 6.2), 72 h, 96%; (e) 2.0 equiv of PMBOC(=NH)CCl_3, 0.1 equiv of CSA, CH_2Cl_2, 48 h, 23 °C, 94%; (f) 1.0 equiv of NaOMe/MeOH, 4 h, 23 °C, 95%; (g) 5.0 equiv of NaH, 3.0 equiv of BnBr, DMF 4 h, 0→23 °C, 93%; (h) 1.2 equiv of DDQ, CH_2Cl_2/H_2O (10:1), 3 h, 23 °C, 85%; (i) 3.0 equiv of pyridine, 2.5 equiv of Tf_2O, CH_2Cl_2, 15 min, −10 °C; then alcohol **9**, in CH_2Cl_2, 2 h; (j) 3.0 equiv of TBAI, benzene, 2 h, reflux, 82% for two steps; (k) 2.5 equiv of PMe_3 (1 M in THF), NH_4OH/pyridine (1:7), 4 h, 23 °C; (l) 3.2 equiv of CBz–Cl, 5.0 equiv of $NaHCO_3$, dioxane/H_2O (1:1), 20 h, 23 °C, 87% for two steps; (m) 5.0 equiv of $(COCl)_2$ in CH_2Cl_2, −60 °C, 10 equiv of DMSO, 1.0 equiv of **11**, 1 h; then 25.0 equiv of Et_3N, 15 min at −20 °C, 30 min at 23 °C, 93%; (n) 7.0 equiv of 13 in THF, 0 °C, 6.9 equiv of LiHMDS, 10 min; then 1.0 equiv of **12** in THF, 3 h, 0→23 °C; (o) 10.0 equiv of CSA, CH_2Cl_2, 16 h, 23 °C, 33% for two steps; (p) 3.0 equiv of $NaClO_2$, 3.0 equiv of NaH_2PO_4, 10.0 equiv of 2-methyl-2-butene, ^{t}BuOH/H_2O (5:1), THF, 3 h, 23 °C, 97%. PMB = 4-methoxybenzyl; CSA = 10-camphorosulfonic acid; 4-DMAP = 4-dimethylaminopyridine; DMSO = methyl sulfoxide; LiHMDS = lithium bis(trimethylsilyl) amide; DDQ = 2,3-dichloro-5,6-dicyano-1,4-benzoquinone; TBAI = tetrabutylammonium iodide; Cbz = carbobenzyloxy.

Initial attempts to introduce the equatorial carbonyl-group at the C-4 position of 2-DOS involved nucleophilic displacement of an axial iodide-functionality with an appropriate acyl-anion equivalent. Thus, treatment of **9** with Tf_2O and pyridine produced the corresponding triflate, which was further reacted with tetrabutylammonium iodide (TBAI) in refluxing benzene to furnish axial iodide **10** in 82% yield for the two steps.[26] Attempts to displace the iodide with different nucleophiles, including potassium cyanide, trimethylsilyl-cyanide,[27] vinyl-magnesium bromide (with or without copper catalysis), $(vinyl)_2Cu(CN)Li_2$[28] and 1,3-dithiane anion resulted in decomposition or elimination.

Similar results were obtained when different nitrogen-protecting strategies (Boc-, Cbz-) were employed, directing us to modify our approach. Alternatively, the desired carbonyl-functionality could be introduced by a Wittig reaction between the corresponding ketone at the 4-position of 2-DOS and the phosphorus ylide produced from (methoxymethyl)triphenylphosphonium chloride and lithium bis(trimethylsilyl) amide, followed by acid-hydrolysis. Since this direct transformation is not compatible with the azide-functionality due to the potential formation of iminophosphorane intermediates, a two-step reduction/Cbz-protection sequence was performed (Staudinger reduction, Cbz-Cl/$NaHCO_3$), furnishing alcohol **11** in 87% overall yield (Scheme 1). Thus, alcohol **11** was oxidized to the desired cyclohexanone **12**, following Swern's protocol, in 93% yield. The Wittig reaction was performed as shown in Scheme 1. After hydrolysis of the resulting enol-ether with CSA in THF/H_2O we unexpectedly obtained the α,β-unsaturated aldehyde **14**,[29] as the result of a simultaneous hydrolysis-elimination sequence, in 33% overall yield. Obviously, the presence of the benzyl ether at the β-position of the newly formed carbonyl group promoted acid-induced elimination. We nevertheless decided to continue with the synthesis of the 2,5-dideoxystreptamine (2,5-dDOS) derivatives since 5-deoxyneamine, a 2,5-dDOS derivative, was shown to also have antimicrobial activity, including potency superior to neamine against two kanamycin-resistant strains.[30] The further oxidation of aldehyde **14** by sodium chlorite afforded the advanced intermediate carboxylic acid **15** in 97% yield.[31]

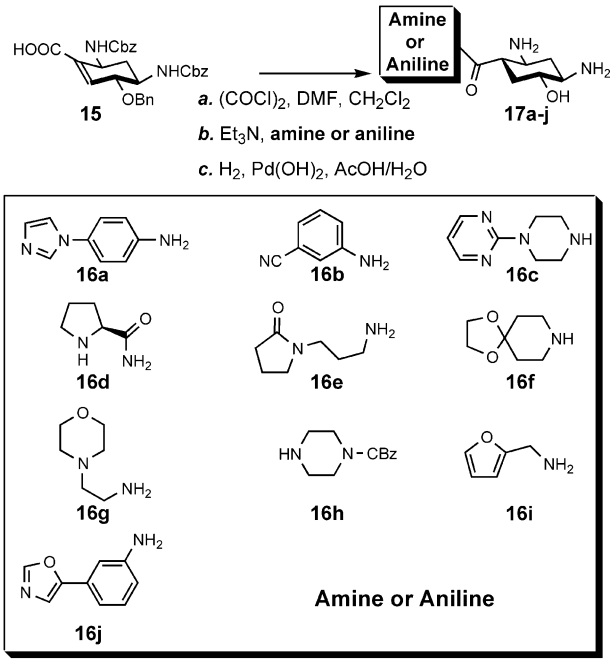
Synthesis of the final amide 2,5-dDOS analogues was performed as outlined in Scheme 2. Thus, carboxylic acid **15** was treated with Vilsmeier salt (DMF/oxalyl chloride) to produce the corresponding acyl-chlorides, which were further reacted with 10 amines (**16a–j**) in the presence of triethylamine, furnishing the desired protected amides. Global deprotection was accomplished by catalytic hydrogenation in acetic acid/water, resulting after concurrent stereoselective saturation of the

Table 1. Structure–activity relationships for 2-DOS derivatives

	Compd	R	IC_{50} (μM)[a]
	**4**		3.9
	5	H	> 1000
	17i	NH_2	> 1000
	17g		> 1000
	17e		> 1000
	17d		> 1000
	**17h**[34]		> 1000
	17c		> 1000
	17f		> 1000
	17b		450
	17a		290[b]
	17j		59[b]

[a]The coupled in vitro transcription-translation assay was carried out in a 384-well plate. The compounds were incubated with bacterial S30 extract (Promega) followed by a mixture containing nucleotide triphosphates, amino acids and pBEST*luc*™ plasmid DNA (Promega) encoding the luciferase reporter. Plates were incubated at 25°C for 20 min. After cooling on ice, SteadyGlow™ luciferin substrate (Promega) was added followed by incubation for 15 min at room temperature. Light emission in the plates was recorded with a TopCount™ (Perkin-Elmer) luminescence counter. Each compound was tested in a dose–response fashion at concentrations ranging from 1 mM to 100 nM. IC_{50} values were determined from light unit versus log(c) plots by fitting to a variable slope dose–response equation. Six replicates were run per concentration. An excellent signal-to-noise ratio was obtained in the assay attested by Z' values[33] in the range of 0.60–0.70 per plate. To rule out that active compounds were inhibitors of the bacterial RNA polymerase or firefly luciferase reporter enzyme, all compounds were counter-screened against polymerase and luciferase. None of the paromamine derivatives inhibited either polymerase or luciferase.

[b]A counter screen suggested that compounds **17a** and **17j** are inhibitors of the luciferase reporter enzyme.



Scheme 2. Reagents and conditions: (a) 3.0 equiv of $(COCl)_2$, 3.0 equiv of DMF, 30 min, −20→0 °C; then 1.0 equiv of acid **15**, 10 min; (b) 1.5 equiv of amine or aniline (**16a**–**j**), 3.0 equiv of Et_3N, 3 h, 0→23 °C; (c) 0.05 equiv of $Pd(OH)_2$, $AcOH/H_2O$ (1:1), 20 h, 23 °C, 82% for **17a**, 66% for **17b**, 72% for **17c**,[32] 36% for **17d**, 34% for **17e**, 30% for **17f**, 92% for **17g**, 74% for **17h**, 41% for **17i**,[32] 21% for **17j** overall; DMF = *N*,*N*-dimethylformamide.

double bond, in the final compounds **17a**–**j** in good to excellent overall yields (Scheme 2).[32]

The biological activity of the compounds was evaluated in a coupled in vitro transcription–translation assay using firefly luciferase as a reporter (Table 1). The translation assay uses a bacterial extract, containing functional ribosomes, to synthesize luciferase protein in vitro. The produced luficerase enzyme is quantified by subsequent addition of luficerin substrate, giving rise to a light signal that is proportional to the amount of functional luciferase present. Inhibitors of translation, such as ribosomal A site RNA-binding compounds, interfere with the production of luciferase enzyme, thereby reducing the observed light emission. Potential inhibition of luciferase itself was subsequently tested in a counter screen in which a predetermined amount of luciferase was incubated with luciferin substrate in the presence of compound.

Three anilides (**17a**, **17b**, and **17j**) inhibited the assay at medium to high micromolar concentrations. Two of the active compounds (**17a** and **17j**) were identified as inhibitors of the luciferase reporter enzyme. Thus, it was not possible to determine their inhibition of translation. The single remaining bona fide inhibitor of bacterial translation, the cyano-anilide (**17b**) was roughly two orders of magnitude less active than the paradigm compound paromamine (**4**). 2-DOS (**5**) itself and the unsubstituted 2,5-dDOS-4-amide (**17i**) did not inhibit the translation assay, revealing the inability of the isolated DOS core to recognize the decoding site RNA despite its conservation in biologically active aminoglycoside antibiotics.

The inactivity of the synthesized 2,5-dDOS-4-amides suggests that the scaffolds linked to the 2,5-dDOS core are not able to substitute for the interactions of the amino-sugar in the active natural aminoglycosides neamine (**3**) and paromamine (**4**). This observation is in line with the previously observed weak binding in the high micromolar range of 2-DOS-4-ether derivatives.[19] Alternatively, the amide linkage between the non-sugar scaffolds and 2,5-dDOS, initially designed to provide increased compound rigidity, may lock the 2,5-dDOS-4-amides in an unfavourable conformation for recognition of the decoding site RNA target. The overall low activity of nonglycosidic 2,5-dDOS derivatives as inhibitors of bacterial translation underlines the exquisitely sensitive structure–function relationship in the aminoglycosides targeting the decoding site RNA, and their privileged combination of three-dimensional structure definition and presentation of polar functionality.[15] This is confirmed by a recently published high-quality crystal structure of paromomycin bound to a decoding site RNA construct,[7] which reveals numerous key interactions between the paromamine core (see Fig. 1) and the RNA, aligning the pyranose ring in a striking Watson–Crick base pair-like fashion with the adenine 1408 base.[7]

While the low activity of the 2,5-dDOS-4-amides rules out their further exploitation as ligands for the bacterial decoding site RNA, the synthesized compounds will be further tested in assays of other RNA targets involved in pathogenic processes. The composition of the described 2,5-dDOS derivatives, obtained by the careful choice of amines used in the coupling, renders them as generally 'RNA-friendly' compounds that are valuable to build an exploratory library suitable for screening against any RNA target of interest.

Acknowledgements

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31. α,β-Unsaturated carboxylic acid **15**: ^{1}H NMR (400 MHz, dimethyl-d_6 sulfoxide) δ 12.5 (s, 1H), 7.58–7.21 (m, 15H), 6.63 (bs, 1H), 5.16–4.93 (m, 4H), 4.63 (d, $J_{A,B}=13.0$ Hz, 1H), 4.55 (bd, $J_{A,B}=13.1$ Hz, 1H), 4.55–4.42 (m, 1H), 4.18–4.05 (m, 1H), 3.72–3.60 (m, 1H), 2.13–1.99 (m, 1H), 1.71–1.58 (m, 1H), ^{13}C NMR (100.1 MHz, dimethyl-d_6 sulfoxide) δ 167.7, 156.6, 156.3, 139.3, 139.0, 138.2, 138.1, 134.7, 129.3, 129.2, 129.1, 128.7, 128.6, 128.6, 128.5, 128.4, 128.4, 77.2, 71.6, 66.2, 66.0, 51.5, 47.3, 31.3.
32. For amines **16c** and **16i** the final hydrogenation step resulted in cleavage of the furane or partial reduction of the pyrazine ring, respectively, leading to the formation of products **17c** and **17i**.
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34. Representative example of the final 2,5-dDOS-4-amides, **17h**: ^{1}H NMR (400 MHz, CD_3OD) δ 3.58–3.45 (m, 4H), 3.34–3.24 (m, 1H), 3.14–3.05 (m, 1H), 2.85–2.54 (m, 6H), 2.01 (dt, $J=4$ Hz, $J=12.4$ Hz, 1H), 1.87 (dt, $J=4$, $J=13.2$ Hz, 1H), 1.34 (q, $J=13.2$ Hz, 1H), 1.24 (q, $J=12.0$ Hz, 1H), LRMS m/z calcd for $C_{11}H_{23}N_4O_3$, (M + H): 243.2, found 243.2.