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Synthesis of triazole-functionalized 2-DOS analogues and their evaluation as A-site binders

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ABSTRACT

Aminoglycoside-antibiotics represent important tools for studying the biological functions of RNA. An orthogonal protection strategy applied on 2-deoxystreptamine (2-DOS) revealed a series of key intermediates that enable its regioselective functionalization. Our approach allowed the construction of selected representatives of triazole-containing analogues with diverse molecular frameworks for biological evaluation regarding their binding and antibacterial potencies.

Our rapidly expanding knowledge of the key biological roles that RNA plays has fueled a growing interest in exploiting RNA as a drug target. The decoding site of the bacterial ribosome, or A-site, an internal loop within the 16S rRNA, is the molecular target for natural aminoglycoside antibiotics (Fig. 1).[1–7] Most of these natural products embody in their structures a highly conserved diaminocyclohexitol, 2-deoxystreptamine (2-DOS, Fig. 1), whose essential role for binding to RNA[5,8–12] involves not only direct hydrogen bonding interactions but also the precise orientation of its peripheral functionalities for increased binding affinity. Thus, classification of 2-DOS as a 'privileged' RNA-targeting chemical entity for the development of novel antiviral and antimicrobial pharmaceuticals is highly substantiated. Some of the most studied sub-families of aminoglycoside antibiotics are presented in Figure 1, including the 4-monosubstituted neamine (neomycin A), the 4,6-disubstitution pattern present in the kanamycin class, the 4,5-disubstituted neomycin series and the 1,4,6-trisubstituted semi-synthetic amikacin.[13] The observed variety in their substitution pattern stimulated several efforts towards the synthesis and utilization of an orthogonally protected 2-DOS intermediate, enabling the obvious opportunity to construct analogues of the natural products through a unified approach from a common precursor.[14–18] Herein we would like to present our findings on that subject, resulting in the synthesis of diverse representative

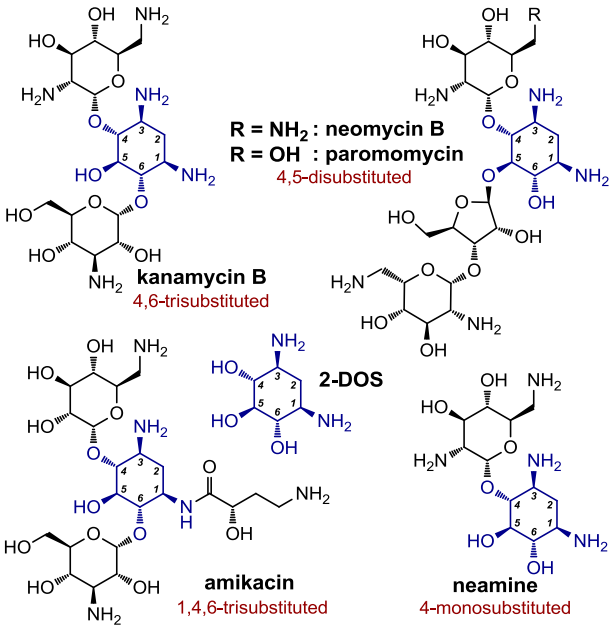

Figure 1. Structures of the 4,5-disubstituted neomycin B and paromomycin, 4-monosubstituted neamine (neomycin A), 2-deoxystreptamine (2-DOS), 4, 6-disubstituted kanamycin B and 1,4,6-trisubstituted semi-synthetic amikacin.

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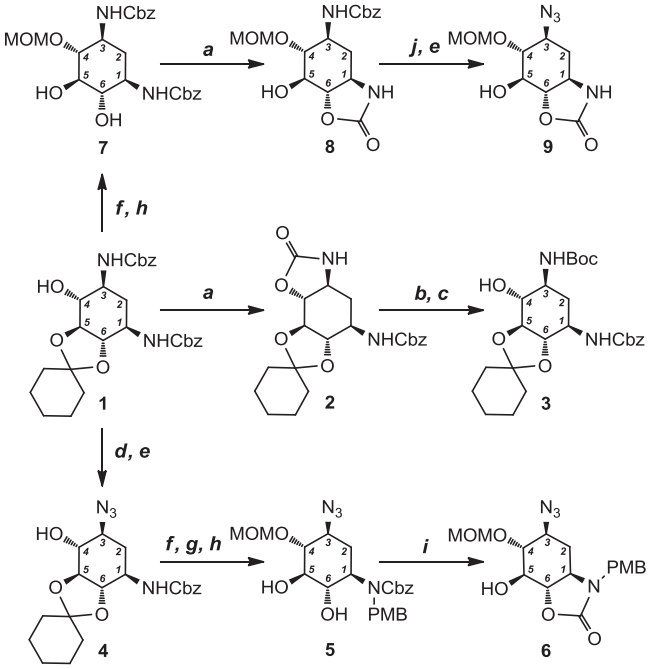
examples of mono-, di- and tri-substituted triazole-containing 2-DOS analogues for further biological evaluation. Our approach was based on existing literature precedent[19–21] and our experience in this field.[22–25] Triazoles were selected primarily due to their previous success in similar systems, as the functionality that dramatically increases binding affinities for the ribosomal decoding site, when placed in the area occupied by position-4 substituents (Fig. 1 numbering).[26] Consequently, adjacent positioning (3 and 5), amplified H-bonding potential and overall rigidity modifications of the final structures were rationally targeted.

Our synthetic approach was initiated from optically pure alcohol **1**.[25] This intermediate was obtained from neamine, exploiting to our advantage the chiral substitution pattern of 2-DOS in the natural products, and thus avoiding chiral auxiliary- or enzymatically-induced resolution strategies. Formation of oxazolidinone **2** under basic conditions, followed by activation of the cyclic carbamate towards hydrolysis through the introduction of a Boc-group, resulted, after basic treatment with LiOH, to the isolation of 2-DOS analogue **3**, with obvious orthogonal diversification of the amino-protecting groups (Scheme 1).

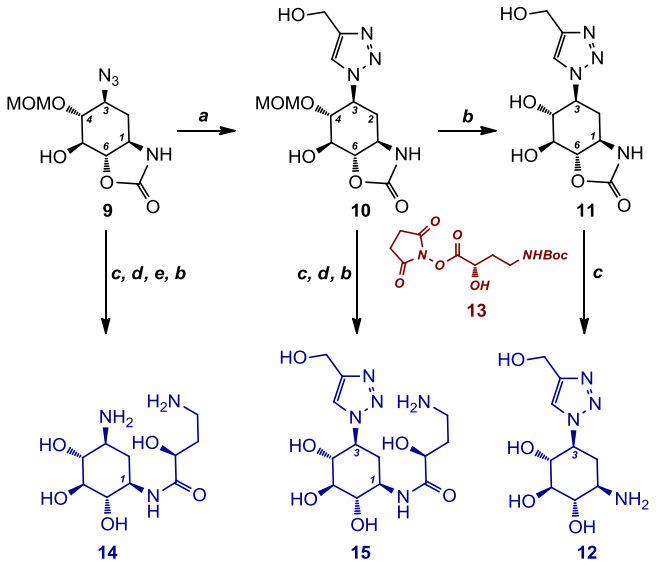
When alcohol **1** was subjected to stronger hydrolyzing conditions, only the amine at position 3 was liberated from the corresponding benzyl carbamate through the neighboring group participation of OH-4, allowing its differential protection as azide **4**.[27] Protection of the free hydroxyl in **4** with MOM-Cl, followed by the introduction of a PMB-group to the acidic oxazolidinone NH (NaH, PMB-Cl) furnished, after selective cleavage of the cyclic ketal, diol **5**. Finally, the two hydroxyls at positions 5 and 6 (Scheme 1 numbering) were differentiated, as before for **2**, by the formation of a cyclic-carbamate, producing orthogonally protected 2-DOS **6**.

The same alcohol could be synthesized by an alternative approach, initiated by etherification of **1** with MOMCl and selective cleavage of the cyclic ketal, to provide diol **7** in good yield. Subsequently, intramolecular oxazolidinone formation under basic conditions furnished alcohol **8**. Hydrogenolysis of the benzyl carbamate and transformation of the liberated amine to the corresponding azide (triflic azide, Cu^{II} catalysis),[27] furnished compound **9**. Finally the desired alcohol **6** resulted in 70% yield from the efficient introduction of a PMB-group to the more acidic oxazolidinone NH after treatment of alcohol **9** with stoichiometric NaH (1 equiv) and PMB-Br (3 equiv) in THF at 45 °C for 4 h. The transformations presented in Scheme 1 along with their products, advanced intermediates **6** and **9**, embody all the required characteristics of an orthogonality concept, allowing the efficient regioselective modification of 2-DOS, as it will be presented for representative examples in the following schemes.

The desired triazole functionality was firstly introduced at position 3 (Scheme 2 numbering) of advanced intermediate **9** by its reaction with propargyl alcohol under standard 'click chemistry' conditions,[28] furnishing **10**. Cleavage of the MOM-protection followed by basic hydrolysis of the oxazolidinone moiety resulted in the formation of 3-mono-substituted product **12** in excellent overall yield. Hydrolysis of the oxazolidinone ring in **10** liberated the amine at position 1, allowing the introduction of additional functionalization. (*S*)-4-Amino-2-hydroxybutyric acid was selected for that purpose, since it represents the side-chain of amikacin as well as a potentially rich source of hydrogen-bonding interactions with the biological target. Reaction of its succinate derivative **13**[29] (Supporting information) with the corresponding amine furnished, after removal of the acid-sensitive protecting groups, 1,3-di-substituted product **15**. Similar chemical transformations (hydrolysis of the oxazolidinone, coupling of the amine with the activated ester, reduction of the azide and cleavage of the MOM-ether) resulted in the isolation of 1-mono-substituted analogue **14** for direct comparison with **12** and **15**. Compound **14** was also efficiently obtained



Scheme 1. Reagents and conditions: (a) NaH (4.0 or 7.0 equiv), DMF (0.06 or 0.1 M), 0.5–2 h, 0 °C or 23 °C, 50% for **2** and 51% for **8**; (b) Boc_2O (1.25 equiv), Et_3N (1.25 equiv), 4-DMAP (0.2 equiv), THF (0.01 M), 16 h, 23 °C, 91%; (c) aq LiOH (0.5 M)/dioxane (1:3), 16 h, 23 °C, 64%; (d) aq LiOH (0.5 M)/dioxane (1:2), 4 h, 70 °C, 91%; (e) triflic azide (3.0 or 5.0 equiv), K_2CO_3 (1.5 equiv) or Et_3N (1.5 equiv), $CuSO_4{\cdot}5H_2O$ (0.1 equiv), $MeOH/H_2O$ (5:1), 18 h, 23 °C, quantitative for **4** and 60% over 2 steps for **9**; (f) MOMCl (10.0 equiv), DIPEA (12.0–14.0 equiv), TBAI (0.1 equiv), CH_2Cl_2 (0.1 or 0.5 M), 16 h, 0 → 23 °C, 95–96%; (g) PMBCl (3.0 equiv), NaH (4.0 equiv), TBAI (0.1 equiv), DMF (0.24 M), 10 h, 0 → 23 °C; (h) $AcOH/H_2O$ (4:1), 1.5–3 h, 23 °C, 60% over 2 steps for **5** and 88% for **7**; (i) NaH (3.0 equiv), DMF (0.02 M), 0.5 h, 0 °C, quantitative; (j) H_2, Pd/C (10% moles), MeOH (0.15 M), 3 h, 23 °C, 92%. MOMCl = Chloromethyl-methyl ether; DIPEA = *N*,*N*-Diisopropyl-ethylamine; 4-DMAP = 4-dimethylamino pyridine; TBAI = Tetra-*n*-butyl ammonium iodide; AcOH = Acetic acid; DMF = Dimethylformamide; Tf = Trifluoromethane sulfonyl; PMB = 4-Methoxybenzyl; THF = Tetrahydrofuran.



Scheme 2. Reagents and conditions: (a) propargyl alcohol (2.0 equiv), $CuSO_4{\cdot}5H_2O$ (0.3 equiv), sodium ascorbate (0.5 equiv), $EtOH/H_2O$ (2:1), 12 h, 23 °C, 96%; (b) 1 M HCl/EtOAc or 1 M HCl/MeOH (1:1), 12 h, 23 °C, 97% for **11**, or 77% over 2 steps for **14**, or 94% for **15**; (c) aq LiOH (0.5 M)/dioxane (1:1), 2.5–12 h, 23 °C, 77% for **12**; (d) **13** (2.1 equiv), satd $NaHCO_3$ (1 mL/mmol), dioxane/H_2O (3:1), 12 h, 23 °C; (e) Me_3P (1 M in THF, 5.0 equiv), NaOH (0.05–1.3 equiv), THF (0.1 M), 12 h, 0 → 23 °C, 92%.

from **3** after a 3-steps, hydrogenolysis—coupling—acidic deprotection, sequence.

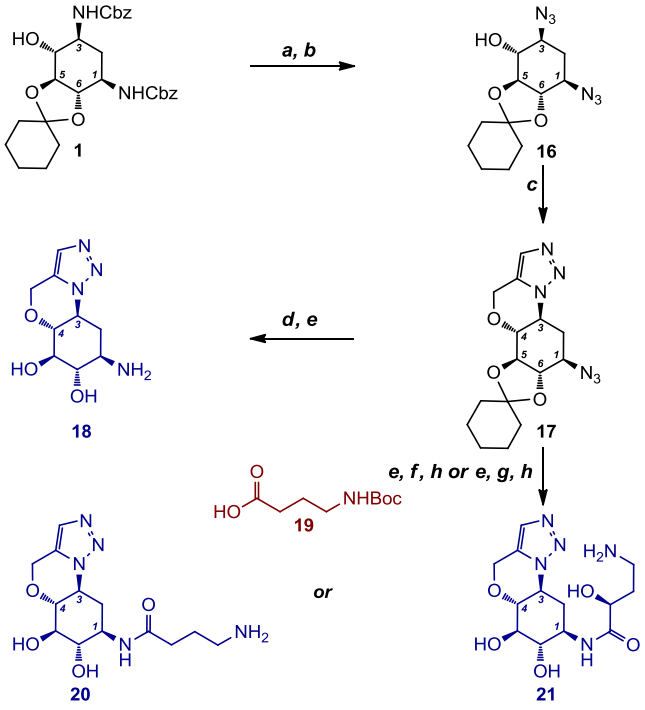
Similar analogues, albeit of increased rigidity, were constructed as presented in Scheme 3. Specifically, replacement of the benzyl carbamates in **1** for azides resulted in the formation of diazido alcohol **16** in 72% overall yield. Alkylation of the 4-OH in **16** with propargyl bromide proceeded with simultaneous cyclization to the corresponding triazole **17**.[30] Cleavage of the ketal protecting group with aqueous AcOH, followed by Staudinger reduction of the azide furnished tricyclic analogue **18** in excellent yield. Additional substitution at position 1 was introduced, either through activated ester **13** (as shown before in the syntheses of **14** and **15**), or by standard amide coupling (EDC) of *N*-protected amino acid **19**, yielding, after removal of the acid-labile protecting groups with HCl, analogues **20** and **21**.

Introduction of triazole-containing moieties at positions 4 or 5 of 2-DOS was performed as presented in Scheme 4. Thus, advanced intermediate **6** was alkylated with propargyl bromide under basic conditions (NaHMDS) furnishing, after liberation of the 4-hydroxyl, alcohol **22**. A second alkylation step at position 4 with α-bromo amide **23** (Supporting information), followed by removal of the PMB-protection, resulted in the formation of **24**. Reduction of the azide and hydrolysis of the oxazolidinone prepared the resulting diamino-alkyne for its reaction with azide **25** under Cu^{I} catalysis[28] furnishing final analogue **26**.

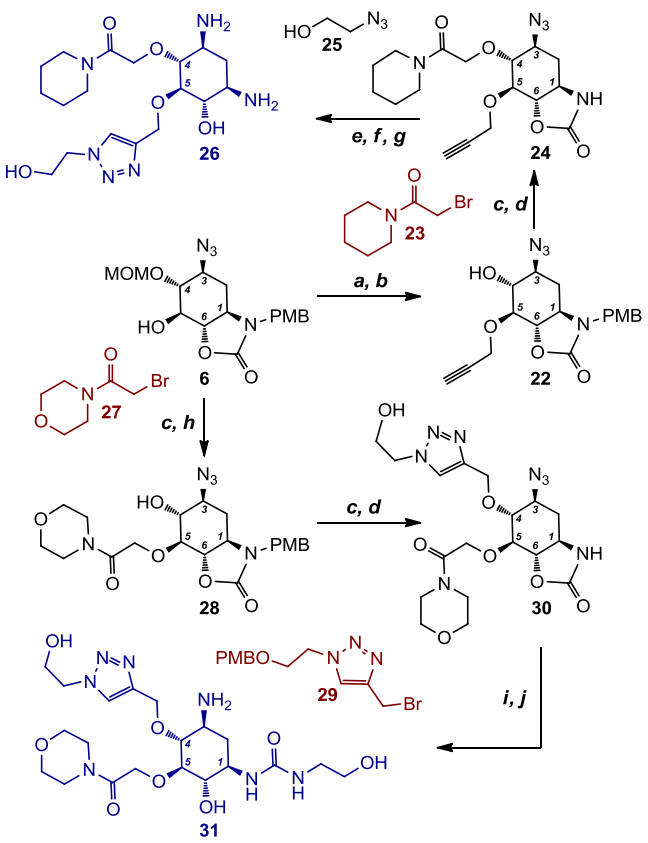
Similarly, alkylation of **6** with bromide **27** (Supporting information) produced after removal of the MOM-protection under acidic conditions, alcohol **28**. Introduction of an alkynyl-moiety on the latter would, as shown before for **17**, lead to an immediate cyclization to the corresponding tricyclic system. To avoid this unwanted reactivity, the desired triazole was in this case pre-formed as bromide **29** (Supporting information) and introduced to the free 4-hydroxyl under basic conditions. Oxidative conditions (CAN) universally cleaved all PMB-protecting groups. Substitution at position 1 resulted from nucleophilic opening of the oxazolidinone with ethanolamine furnishing the corresponding urea. Hydrogenation of the azide moiety completed the synthesis of tri-substituted analogue **31**.

Evaluation of the binding specificity of our analogues for the ribosomal decoding site was performed, as previously described,[30] by an RNA fluorescence assay, based on enhancement of the fluorescence emission of 2-aminopurine (2AP) attached instead of the flexible adenine A1492 of the model oligonucleotide (Table 1, RNA Specific).[31] The activity of the compounds as functional bacterial-translation inhibitors was evaluated in a coupled in vitro transcription-translation assay (IVT, Table 1).[32] The synthetic analogues were also evaluated regarding their antibacterial activity against Gram positive *Staphylococcus aureus* (ATCC 29213) as well as Gram negative *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) strains (Table 1).

Based on the obtained results we speculate that the overall profile of the synthetic analogues represent a composite of various factors. Some of the more important ones dictating their behaviour include: (a) H-bonding and/or π-stacking potential, (b) availability



Scheme 3. Reagents and conditions: (a) H_2, Pd/C (10% mol), MeOH (0.1 M), 12 h, 23 °C; (b) triflic azide (10 equiv), Et_3N (20 equiv), $CuSO_4{\cdot}5H_2O$ (0.05 equiv), MeOH/H_2O (1.5:1), 16 h, 23 °C, 72% over 2 steps; (c) propargyl bromide (80% wt. in toluene, 1.1 equiv), NaH (5.0 equiv), TBAI (0.1 equiv), toluene (0.07 M), 12 h, 0 → 23 °C, 88%; (d) AcOH/H_2O (4:1), 12 h, 23 °C; (e) Me_3P (1 M in THF, 5.0 equiv), aq NaOH (0.1 M, 0.05 equiv), THF (0.1 M), 12 h, 0 → 23 °C, 96% over 2 steps for **18**; (f) **19** (3.0 equiv), EDC (3.0 equiv), DIPEA (4.0 equiv), 4-DMAP (1.1 equiv), CH_3CN (0.3 M), 12 h, 0 → 23 °C, 68% over 2 steps; (g); **13** (2.1 equiv), satd $NaHCO_3$ (1 mL/mmol), dioxane/H_2O (3:1), 12 h, 23 °C; (h) HCl (4 M)/EtOAc (1:1), 12 h, 23 °C, 79% for **20** and 73% for **21** over 3 steps. EDCI = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide hydrochloride.



Scheme 4. Reagents and conditions: (a) Propargyl bromide (3.0 equiv), NaHMDS (1.2 equiv), TBAI (0.1 equiv), THF (0.1 M), 12 h, −4 → 23 °C; (b) HCl (1.4 M in 1:1 MeOH/$CHCl_3$), 12 h, 23 °C, 52% over 2 steps; (c) **23** or **27** or **29** (1.5–2.5 equiv), NaHMDS (1.1 equiv), THF (0.05–0.1 M), 20 min. to 3 h, −4 → 23 °C, 83–96%; (d) CAN (5.0 equiv), CH_3CN/H_2O (9:1), 1–4 h, 23 °C, 75% over 2 steps for **24**; (e) Me_3P (1 M in THF, 3.0 equiv), aq NaOH (1 M, 1.3 equiv), THF (0.04 M), 12 h, 0 → 23 °C; (f) aq LiOH (0.5 M)/dioxane (1:1), 12 h, 23 °C; (g) **25** (2.0 equiv), $CuSO_4{\cdot}5H_2O$ (0.3 equiv), sodium ascorbate (0.5 equiv), EtOH/H_2O (2:1), 12 h, 23 °C, 46% over 3 steps for **26**; (h) concd HCl (cat.), MeOH (0.05 M), 0.5 h, 50 °C, quantitative; (i) ethanolamine (10 equiv), satd $NaHCO_3$ (1.5 equiv), dioxane (0.03 M), 12 h, 23 °C, 72%; (j) H_2, Pd/C (10% mol), MeOH (0.1 M), 4 h, 23 °C, 51%. NaHMDS = sodium bis(trimethylsilyl)amide; CAN = cerium(IV) ammonium nitrate.

Table 1
Binding affinities and biological activities of synthetic analogues

Entry	Compound[a]	RNA Specific[b] (EC_{50}, μM)	IVT[c] (IC_{50}, μM)	*Escherichia coli* (MIC)[d]	*Staphylococcus aureus* (MIC)[d]	*Pseudomonas aeruginosa* (MIC)[d]
1	**12**	No activity	468	>700	88	>700
2	**14**	8.7	475	>840	52.5	>840
3	**15**	No activity	66.5	>1000	60	>1000
4	**18**	53	317	283	>565	283
5	**20**	22.4[e]	55.5	>870	109	>870
6	**21**	0.017[e]	104	>820	102	>820
7	**26**	3	730	>1000	129	>1000
8	**31**	62	22.6	235	78	157

[a] All final products were quantitated by NMR spectroscopy, utilizing an internal standard (DiMethylFuran, DMFu).[33]
[b] RNA-ligand EC_{50} values were determined by decoding-site RNA fluorescence assay (average of 3 replicate experiments per compound, ±10%).
[c] Bacterial in vitro transcription/translation (IVT) IC_{50} values were determined by coupled transcription-translation assay using *E. coli* extract (2 replicate experiments, in duplicate, per compound, ±14%).
[d] Concentrations are reported in μg/mL.
[e] Small efficacy in fluorescence intensity increase effect.

of the two amino-groups at positions 1 and 3 to participate into 'neamine-like' interactions with the A-site (with C1407–G1494 and the non-Watson–Crick pair U1406–U1495),[12] docking the compounds into a preferred conformation, (c) binding orientation adapted within the binding cavity, (d) existence of alternative biological targets (different topologies of binding on the ribosome, other RNAs, other proteins).

Specifically, for analogues **12** and **15** we observe the absence of binding affinity for the bacterial decoding site. Apparently, positioning of the triazole-functionality in a perpendicular orientation to the diaminocyclohexitol, along with the unavailability of N-3 to participate in H-bonding, diminish their potential to participate in effective interactions, rendering these compounds incapable to assume the required binding orientation. Removal of the triazole moiety in **14** re-establishes its binding ability. Thus, according to our previous discussion, the observed inhibition in protein production (IVT for the di-substituted **15**) as well as their potential to inhibit the growth of Gram positive bacterial strains (for **12**, **14** and **15**) are the results of an alternative, unknown at this point, mechanism of action.

When the triazole is forced to adapt a more planar orientation with respect to the 2-DOS ring, **18**, **20** and **21** regain their binding affinity for the A-site, in an analogous manner to their H-bonding potential. This effect is also directly translated into biological activity (IVT and MICs). Small variations in the absolute values of their potencies, comparing either analogues within the same series (**12** to **15** or **18** to **20** and **21**) or different series (**12**, **15** vs. **18**, **20** and **21**, respectively), appear to be directed by the extent of their H-bonding potential and/or differential mechanisms of action and binding orientations.

Same parameters might also dominate the differences between binding potential and biological function of analogue **26** in comparison to **31**. In this case, the superiority of **26** over **31**, regarding its affinity for the decoding center, is completely reversed in their IVT values. Also, **31** is recognized to possess a broad antibacterial activity, affecting both Gram positive and Gram negative microorganisms.

In conclusion, exploiting a series of chemical transformations and protecting groups, we have accomplished the design and synthesis of orthogonally protected 2-DOS advanced intermediates **3**, **6** and **9**. These compounds allowed us to synthesize and evaluate representative examples of triazole-containing analogues with diversified substitution patterns, in terms of topology and functionality. Promising leads for A-site binders, protein production inhibitors and antimicrobials were thus identified, providing at the same time useful indications regarding the complexity of the biological target and its function. The generality of our approach could potentially allow the application of refined medicinal chemistry protocols, by harnessing the molecular diversity accessible to synthetic chemistry. Thus, novel specific RNA binders could be discovered within a new molecular framework, not hampered by complexity.

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Supplementary data

Supplementary data (experimental procedures and NMR spectra of all intermediates and final products along with titration curves) associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2013.12.125.

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