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Second generation analogs of rigid 6,7-spiro scaffolds targeting the bacterial ribosome

Christos I. Stathakis, Ioannis Mavridis, Georgia Kythreoti, Athanasios Papakyriakou, Ioannis A. Katsoulis, Thomas Cottin, Panoula Anastasopoulou, Dionisios Vourloumis *

Laboratory of Chemical Biology of Natural Products and Designed Molecules, Institute of Physical Chemistry, NCSR 'DEMOKRITOS', GR-15310 Ag. Paraskevi Attikis, Athens, Greece

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ABSTRACT

Previous work from our group described the synthesis and biological evaluation of new rigid, 6,6- and 6,7-spiro aminoglycosidic scaffolds targeting the bacterial ribosome. Herein we describe an improved synthetic protocol for their construction, and extend our study by further amino-functionalization of their 6,7-spiro analogs. The synthetic strategy, preparation and evaluation of some representative examples are reported.

The ribosomal RNA (rRNA) of bacteria is well recognized as the actual target for many clinically useful antibiotics.[1] Aminoglycosides (Fig. 1A) occupy a unique space alongside other RNA-binding drugs, especially due to their exceptional ability to bind on the decoding site (A-site) (Fig. 1B) within the 16S subunit of bacterial ribosome, interfering with the process of protein production.[2] Along with specific toxicity issues, aminoglycoside antibiotics suffer from development of resistance, especially due to their extensive use, facts that significantly limit their therapeutic potential.[3] Nevertheless, their capacity to bind with high affinity to the bacterial decoding site and several other RNA targets[4] renders them a lead paradigm in RNA molecular recognition and ideal starting points for the design and synthesis of novel RNA binders.

Previous research efforts from our group revealed a new series of spiro bicyclic scaffolds (Fig. 1C), which successfully mimicked the natural products in their ability to specifically bind the ribosomal decoding center.[5] In that work, we hypothesized that the introduced rigidity would 'lock' the specific analogs in the desired bioactive conformation, while their decoration with appropriately positioned amino- and hydroxyl-functionalities would enhance their binding ability. Thus, 6,6- and 6,7-spiro compounds were designed, and synthesized. Their biological properties were evaluated in terms of binding on specific RNA subunits as well as in vitro inhibition of protein synthesis in bacterial and eukaryotic systems.[5]

Stimulated by the promising results of the parental spiro-compounds we anticipated that further functionalization of these structures could lead to improved biological profiles. Amikacin's unique substitution pattern and exceptional antibacterial properties,[6] along with examination of its predicted binding orientation, directed us to initially concentrate on N1 (DOS numbering, Fig. 1A). Moreover, our current study would be performed on the bulkier 6,7-bicyclic analogs, since they could more efficiently occupy the binding cavities of both, the bacterial and the eukaryotic constructs.

From a retrosynthetic perspective, we envisioned the required N1 versus N3 differentiation to be the outcome of a selective oxazolidinone formation, as presented in Scheme 1. Specifically, we would take advantage of the immediate proximity of N-1 to the free 6-hydroxyl, which under basic conditions would lead to a nucleophilic attack on the adjacent carbamate group, furnishing intermediate structure **III**.[7,5] Consequently, oxazolidinone **III** could be selectively hydrolyzed under controlled basic conditions to amine **II**, which could be further functionalized through reductive amination or acylation transformations, delivering the desired analogs **I**. In turn, the required precursor **IV** would be the outcome of an olefin metathesis reaction from diene **V** as presented before.[5] Regarding positions 3′ and 4′ of the seven-membered ring (Scheme 1), our docking calculations indicated that both, hydrogen bonding interactions or π-stacking, were probable. Thus, retention of the olefin or dihydroxylation were equally considered for exploitation purposes. Additionally, the stereochemistry for the dihydroxylation reaction was of minor importance at this time, since similar

* Corresponding author. Tel.: +30 210 650 3624; fax: +30 210 651 1766.
E-mail address: vourloumis@chem.demokritos.gr (D. Vourloumis).

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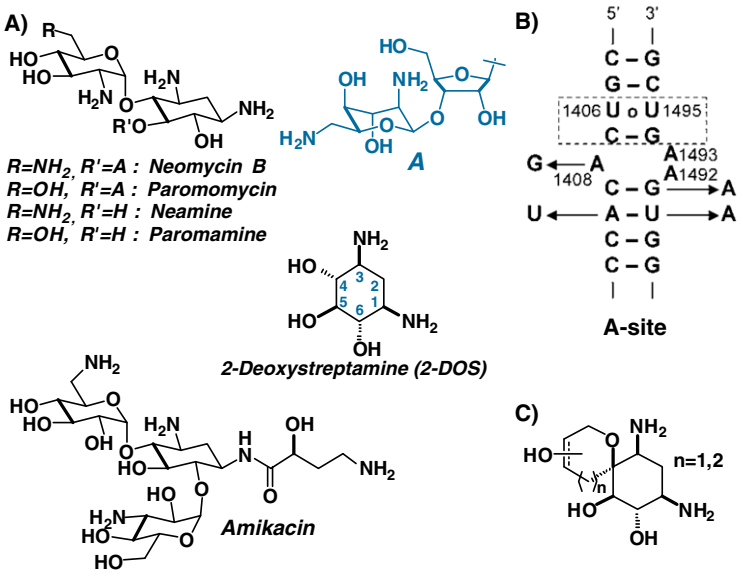


Figure 1. (A) Selected examples of aminoglycosides with antibiotic activity. The common feature, 2-deoxystreptamine (2-DOS) core is also presented; (B) Secondary structure of the bacterial decoding site (A-site) in 16S rRNA. The four base-changes of the eukaryotic sequence are indicated by arrows. The recognition site for the 2-DOS moiety of aminoglycosides is boxed; (C) New spiro bicyclic analogs demonstrating good affinities for the decoding site of bacterial ribosome.[5]

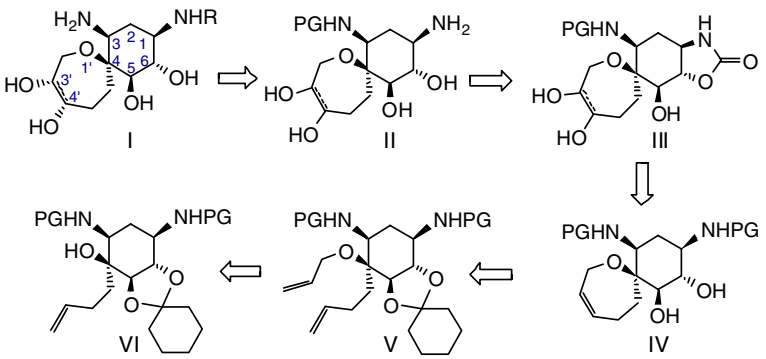
hydrogen bonding potential with the RNA was predicted for the two isomers.

It is important to emphasize that in our previous efforts we were forced to exchange the amino-protecting carbamates, required in this study, to the corresponding acetamides, in an attempt to overcome the inherent tendency of **1** to react intramolecularly producing the corresponding oxazolidinone **2** (Scheme 2).[5] Also, the dihydroxylation transformation of the specific series (6,7-spiro) was shown to be non-selective (3:2 d.r.) regardless of the use of chiral ligands or low temperatures (0 °C).[5]

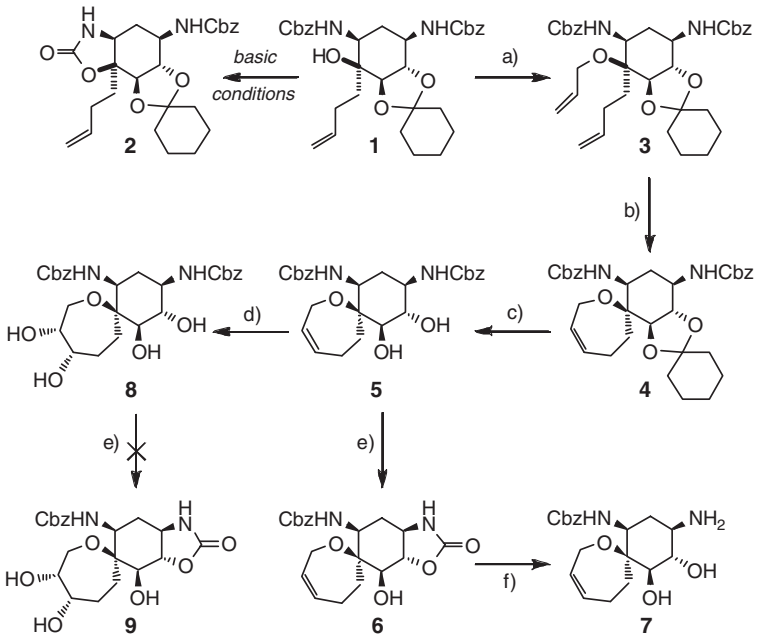
With the intention of exploring the viability of the suggested strategy, we initiated our study from tertiary alcohol **1** (>8:1 d.r).[5] The first critical transformation was, as described before, the allylation of **1**, under neutral conditions, so as to avoid the formation of the undesired oxazolidinone. After numerous unsuccessful trials, involving basic (NaH, DIPEA, LiHMDS, Ag_2O) or acidic conditions (allyltrichloroacetamidate and *p*-TsOH or TfOH or $Sc(OTf)_3$), the anticipated allylation to ether **3** was accomplished in very good yield (88%) utilizing allyl-*t*-butylcarbonate as the allyl-donor in the presence of catalytic amounts of $Pd(PPh_3)_4$.[8]

Ring closing metathesis (RCM) was performed in almost quantitative yield, utilizing Grubbs' second generation catalyst,[9] furnishing the corresponding spiro alkene **4** as a single isomer (C-4). The minor diastereomer was never isolated after the final chromatographic purification. Upon cleavage of the protecting ketal under acidic conditions, diol **5** was accessed, which was next utilized for testing the aforementioned hypothesis for amine-differentiation. Indeed, treatment of diol **5** with NaH in DMF,[7] produced the expected oxazolidinone **6** in very good yield (78%). Subsequently, treatment of oxazolidinone **6** with 0.5 N LiOH in dioxane could selectively hydrolyze the more labile *trans*-fused cyclic urethane, in comparison to the Cbz-carbamate, providing access to the anticipated diol-amine **7**. In parallel, dihydroxylation of **5** to tetrol **8** was accomplished with excellent stereoselectivity, utilizing the conditions presented in Scheme 2.[10] Subsequent exposure of **8** to the same conditions governing the formation of **6**, led to an unexpected complicated mixture of products. Consequently, the dihydroxylation reaction would be performed as the penultimate step of our approach.

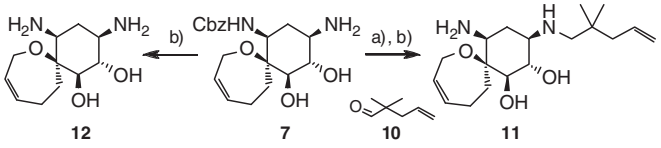
With the appropriate precursor in hand, our derivatization plan was realized by means of two distinct protocols. The first involves a reductive amination condensation with various aldehydes, giving rise to more complex secondary amines. For illustration of its potential, the sterically demanding and highly lipophilic aldehyde **10** was selected, which under standard conditions ($NaBH_3CN$, cat. AcOH in MeOH) furnished the corresponding secondary amine in



Scheme 1. Strategy for the differentiation of the amino groups on the amino- cyclitol moiety. PG = carbamate group.



Scheme 2. Reagents and conditions: (a) Allyl-*t*-butylcarbonate (3.0 equiv), $Pd(PPh_3)_4$ (0.1 equiv), THF, 45 °C, 88%; (b) Grubbs 2nd generation (5 mol %), CH_2Cl_2 (0.01 M), 25 °C, 12 h, 91%; (c) THF/AcOH/H_2O, 3:3:1, 50 °C, 8 h, 87%; (d) OsO_4 (1.2 equiv, 2.5% wt in *t*-BuOH), TMEDA (1.2 equiv), CH_2Cl_2, −78 °C, 30 min, 69%, single diastereomer; (e) NaH (4.0 equiv, 60% in mineral oil), DMF, 25 °C, 4 h, 78%; (f) 0.5 N LiOH (5.0 equiv), dioxane, 25 °C, 12 h, 74%. AcOH = acetic acid; DMF = dimethylformamide; THF = tetrahydrofuran; TMEDA = *N*,*N*,*N*′,*N*′-tetramethylethylenediamine; TMSCl = trimethylchloro silane.

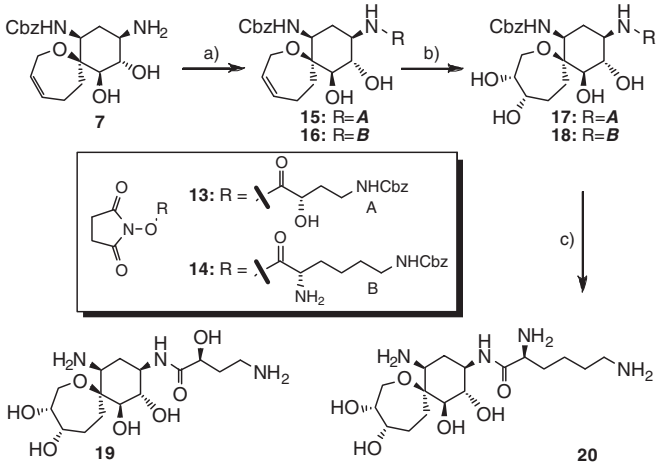


Scheme 3. Reagents and conditions: a) $NaBH_3CN$ (2.5 equiv), AcOH (0.2 equiv), aldehyde **10** (1.1 equiv), MeOH, 25 °C, 8 h, 61%; b) KOH (40.0 equiv), MeOH/H_2O 1:1, 95 °C, 12 h, 78% for **11** and 82% for **12**.

61% yield (Scheme 3). After basic hydrolysis of the Cbz-carbamate, functionalized analog **11**[11] was isolated for further biological evaluation. Similar hydrolysis conditions applied on **7** produced the parent unsaturated compound **12**[12] for direct comparison. Both analogs would serve us to evaluate the extent of lipophilicity required for retention of a reasonable binding affinity to the charged RNA target.

The second protocol incorporates an amide coupling with activated forms of functionalized carboxylic acids. Following a comprehensive screening protocol for identifying the ideal coupling method for the specific transformation (DCC, DCC/4-DMAP, EDC/HOBt, HATU, ^{t}Bu-mixed anhydrides), it was found that the succinate derivatives of the corresponding carboxylic acids represented the best coupling partner.[7] Two examples were selected, one involving the L-hydroxyaminobuteroyl group (amikacin's side chain) and the other a lysine, both introducing an extensive hydrogen bonding potential to our analogs. Thus, reactions of the corresponding dicarboximides **13** and **14** with diol-amine **7** were performed, furnishing amides **15** and **16** in good yields (Scheme 4). Both amides were subjected to the pre-described dihydroxylation conditions (OsO_4, TMEDA),[10] producing stereoselectively tetraols **17** and **18**. Two-dimensional NMR experiments on the fully deprotected derivatives **19** and **20**,[13,14] formed after hydrogenolysis of the corresponding Cbz-carbamates, confirmed their stereochemistry as the one depicted in Scheme 4.

In order to examine the specificity of our analogs for the ribosomal decoding site, an RNA fluorescence assay was employed,

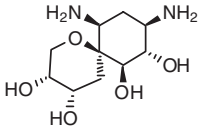
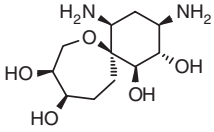
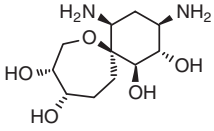


Scheme 4. Reagents and conditions: (a) succinate **13** or **14** (2.1 equiv, 0.1 M in THF), $NaHCO_3$, dioxane/H_2O 3:1, 25 °C, 5 h, 58% for **15**, 78% for **16**; (b) OsO_4 (1.2 equiv, 2.5% wt in *t*-BuOH), TMEDA (1.2 equiv), CH_2Cl_2, −78 °C, 30 min, 63% for **17**, 70% for **18**; (c) 10% Pd/C, 1,3-cyclohexadiene (50.0 equiv), AcOH (0.1 M), 25 °C, 4 h, 82% for **19**, 68% for **20**.

based on enhancement of the fluorescence emission of 2-aminopurine (2AP) attached to the flexible adenine A1492 of the model oligonucleotide (Table 1, RNA fluorescence assay).[15] The biological activity of the compounds as functional bacterial-translation inhibitors was evaluated in a coupled in vitro transcription–translation assay (IVT; Table 1, Bacterial IVT).[16] Additionally, we investigated the performance of these compounds towards eukaryotic protein translation inhibition in vitro (Table 1, E_{IVT}).[5] Table 1 incorporates our results in comparison to the values obtained previously for two natural aminoglycosides and the parent 6,6- and 6,7-bicyclic analogs.[5]

In an attempt to comprehend and, more importantly, 'visualize' our results for further development, we employed docking

Table 1
Biological activities for natural aminoglycosides and spiro derivatives

Entry	Compound[a]	RNA EC_{50}[b,c]	BIVT IC_{50}[b,d]	EIVT IC_{50}[b,e]
1	Neomycin B	0.007	0.032	252
2	Neamine	9.5	5.0	120
3		6.5	11.3	>1000
4		141	168	160
5		74	118	130
6	**12**	(8.4)[f]	>1000	>1000
7	**11**	63	429	>1000
8	**19**	6.9	186	>1000
9	**20**	12	98	>1000

[a] All final products were quantitated by NMR spectroscopy, utilizing an internal standard (DiMethylFuran, DMFu).[17]
[b] Concentrations are reported in μM.
[c] RNA-ligand EC_{50} values were determined by decoding site RNA fluorescence assay (average of three replicate experiments per compound, ±10%).
[d] Bacterial in vitro transcription/translation (B_{IVT}) IC_{50} values were determined by coupled transcription-translation assay using *E. coli* extract (average of three replicate experiments per compound, ±15%).
[e] Eukaryotic in vitro transcription/translation (E_{IVT}) IC_{50} values were determined by the TNT quick coupled transcription/translation assay using reticulocyte lysate (average of three replicate experiments per compound, ±15%).
[f] Low efficacy in fluorescence-intensity effect (see Supplementary data).

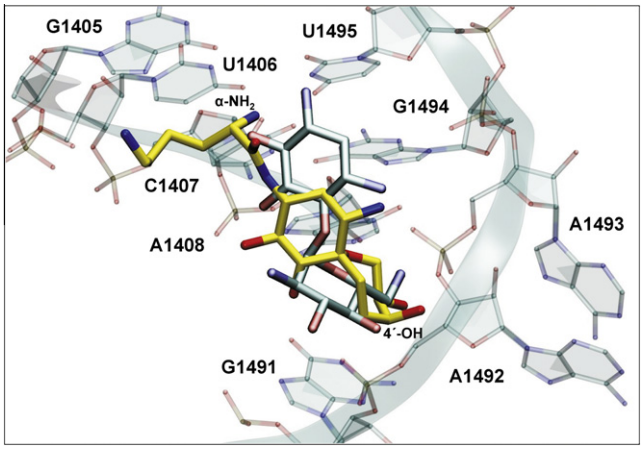


Figure 2. Predicted binding orientation of compound **20** (yellow stick carbon chain) superimposed with the crystallographic position of neamine (cyan stick carbons) docked at the bacterial A-site. Specific interactions predicted include: (a) the 4′-OH with the phosphate backbone of A1492, (b) the α-NH_2 with either U1406 or the U1406○U1495 base pair, respectively, (c) the terminal NH_2 with the phosphates of G1405 or U1406 and (d) the amide-H with the C1407○G1494 pair. Nitrogen atoms are shown in blue and oxygen atoms in red.

calculations as previously described.[5] Although the absence of a co-crystal structure renders this method highly speculative, important interactions may be identified in the process, directing future synthetic endeavors. As a general trend, the obtained binding orientation of the new entities was slightly altered, with N-3 (DOS numbering, Fig. 1) maintaining its position relatively to neamine, while the rest of the skeleton is rotated counterclockwise enabling direct interaction of the ring-oxygen with N6 of A1408, as presented in Figure 2.

Compound **12** represents, as shown by its biological evaluation, a noteworthy entry in the specific series. Its unexpected binding affinity, calculated based on a small fluorescence-intensity range (see Supplementary data) might be indicative of a low efficacy binding orientation. The latter is affecting only a minor conformational change, not sufficient to observe the analogous biological effect. Apparently, the potential of π-stacking interactions between the 3′,4′-double bond and G1491 cannot compensate for the loss of the two hydroxyls relatively to the parent compounds (entries 4, 5, 6, Table 1). Introduction of a lipophilic side chain sufficiently recovers some of its biological activity, as indicated by direct comparison between **11** and **12**. Major improvement in the binding potential was observed by the introduction of hydrophilic chains, as exemplified in **19** and **20** (entries 8 and 9). Anticipated interactions of these compounds with the target RNA are presented in (Fig. 2). Interestingly, the overall effect of these analogs in the inhibition of bacterial protein translation does not follow the same trend (entries 4 and 5 vs 7, entry 8 vs 9).

In conclusion, the development of a new synthetic approach enabled the construction of four novel 6,7-spiro bicyclic aminoglycoside mimics. Our improved synthetic protocol resolved previous issues for the introduction of an allyl group on a tertiary hydroxyl by utilizing Pd-chemistry performed under neutral conditions. Consequently, the retention of the protecting carbamates for the amines present enabled the differentiation of N1 for further functionalization, through the formation of a *trans*-oxazolidinone intermediate. Also, careful selection of the dihydroxylation conditions delivered our final analogs with complete stereocontrol. Overall, a significant improvement of the binding affinities for the bacterial ribosome's decoding center was achieved, in comparison to the parent compounds.[5] Furthermore, we observed that a direct correlation between binding affinities and in vitro biological potency is not always possible. Application of our findings to other bicyclic scaffolds and further mechanistic investigations are currently underway.

Acknowledgments

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Supplementary data

Supplementary data associated (detailed description of the biological methods) with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.10.001.

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11. Compound **11**: R_f = 0.3 in 50% MeOH/CH_2Cl_2; ^{1}H NMR (500 MHz, MeOH-d_4) δ 5.84–5.94 (m, 1*H*), 5.73 (d, *J* = 9.4 Hz, 1*H*), 5.58 (d, *J* = 9.4 Hz, 1*H*), 5.04 (d, *J* = 15.8 Hz, 1*H*), 5.01 (d, *J* = 11.4 Hz, 1*H*), 4.38 (d, *J* = 16.9 Hz, 1*H*), 4.24 (d, *J* = 16.9 Hz, 1*H*), 4.13 (br s, 1*H*), 3.84 (br s, 1*H*), 3.74 (d, *J* = 14.3 Hz, 1*H*), 3.67 (br s, 1*H*), 3.46 (br s, 1*H*), 2.59 (d, *J* = 14.3 Hz, 1*H*), 2.11–2.38 (m, 3*H*), 1.94–2.07 (m, 2*H*), 1.87–1.93 (m, 3*H*), 0.92 (br s, 6*H*); ^{13}C NMR (125 MHz, MeOH-d_4) δ 136.4, 132.2, 129.7, 117.6, 80.0, 73.9, 71.1, 61.9, 56.7, 50.9, 46.6, 34.5, 26.2, 26.0, 25.1, 24.2, 23.0. HRMS-ESI (*m*/*z*) [M+Na]$^+$ calcd for $C_{18}H_{32}N_2NaO_3$ 347.2311, found 347.2313.
12. Compound **12**: R_f: 0.73 (NH_4OH(aq)/MeOH, 1:9 v/v); ^{1}H NMR (D_2O; 500 MHz) δ 5.74 (br s, 1*H*), 5.60 (br s, 1*H*), 4.31 (d, *J* = 16.4 Hz, 1*H*), 4.21 (d, *J* = 16.4 Hz, 1*H*), 4.04 (br s, 1*H*), 3.87 (br s, 1*H*), 3.73 (br s, 1*H*), 3.55 (br s, 1*H*), 3.46 (br s, 1*H*), 3.28 (br s, 1*H*),2.20 (br s, 1*H*), 2.15 (d, *J* = 14.5 Hz, 2*H*), 2.07–2.03 (m, 1*H*), 1.95–1.89 (m, 1*H*), 1.86–1.81 (m, 2*H*), 1.53 (br s, 1*H*); ^{13}C NMR (D_2O, 125 MHz) δ 131.8, 128.3, 78.4, 73.1, 72.3, 60.5, 48.7, 47.3, 33.0, 23.6, 20.8; HRMS-ESI (*m*/*z*) [M+H]$^+$ calcd for $C_{11}H_{20}N_2O_3$ 228.1474, found 228.1471.
13. Compound **19**: R_f = 0.12 in MeOH/CH_2Cl_2/$NH_4OH_{(aq.)}$, 10/30/1 v/v; ^{1}H NMR (500 MHz, D_2O) δ 4.51 (d, *J* = 12.9 Hz, 1*H*), 4.34 (dd, *J* = 7.2, 3.5 Hz, 1*H*), 3.73–3.91 (m, 5*H*), 3.41 (dd, *J* = 9.5, 2.0 Hz, 1*H*), 3.37 (d, *J* = 9.4 Hz, 1*H*), 3.17 (m, 2*H*), 2.54 (dd appt, *J* = 13.6 Hz, 1*H*), 2.15–2.20 (m, 1*H*), 2.01–2.11 (m, 2*H*), 1.92–2.00 (m, 1*H*), 1.75–1.89 (m, 3*H*); ^{13}C NMR (125 MHz, D_2O) δ 175.4, 77.7, 77.2, 74.6, 71.6, 71.3, 69.5, 64.8, 55.7, 49.2, 36.5, 30.7, 30.0, 27.4, 26.6. HRMS-ESI (*m*/*z*) [M+Na]$^+$ calcd for $C_{15}H_{29}N_3NaO_7$ 386.1903, found 386.1900.
14. Compound **20**: R_f = 0.15 in MeOH/CH_2Cl_2/$NH_4OH_{(aq.)}$, 10/30/1 v/v; ^{1}H NMR (500 MHz, D_2O) δ 4.54 (d, *J* = 13.0 Hz, 1*H*), 4.04 (t, *J* = 6.5 Hz, 1*H*), 3.85–3.95 (m, 3*H*), 3.75–3.82 (m, 2*H*), 3.46 (dd, *J* = 11.1, 5.0 Hz, 1*H*), 3.40 (d, *J* = 10.1 Hz, 1*H*), 3.04–3.09 (m, 2*H*), 2.57 (t, *J* = 13.3 Hz, 1*H*), 1.76–2.08 (m, 9*H*), 1.47–1.55 (m, 2*H*); ^{13}C NMR (125 MHz, D_2O) δ 169.5, 77.6, 77.1, 74.6, 71.6, 71.5, 64.8, 55.6, 53.2, 49.6, 38.9, 30.2, 30.1, 27.3, 26.6, 26.3, 21.3. HRMS-ESI (*m*/*z*) [M+Na]$^+$ calcd for $C_{17}H_{34}N_4NaO_6$ 413.2376, found 413.2375.
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