Efficient Synthesis of Heterocyclic 2-Deoxysteptamine Derivatives as RNA Binding Ligands

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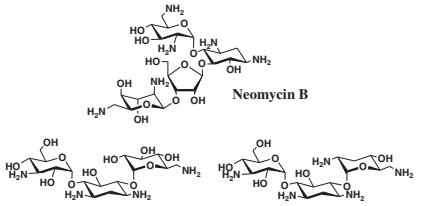
An efficient strategy for the synthesis of heterocyclic 2-deoxystreptamine derivatives is described. The resulting compounds showed good RNA binding affinities from ESI-MS RNA binding assay.

The aminoglycoside antibiotic neomycin B has been demonstrated to inhibit the association of the HIV-encoded Rev-protein with a specific sequence of viral RNA termed the Rev response element.[1] Other aminoglycoside antibiotics such as kanamycin A and tobramycin have been shown to inhibit the binding of a Tat-derived peptide to TAR RNA.[2] By comparing these antibiotics, it is very interesting that all of them are substituted at the 4- and 5-position of the central 2-deoxystreptamine core, we sought this core structure was capable of providing a collection of diverse RNA ligands.



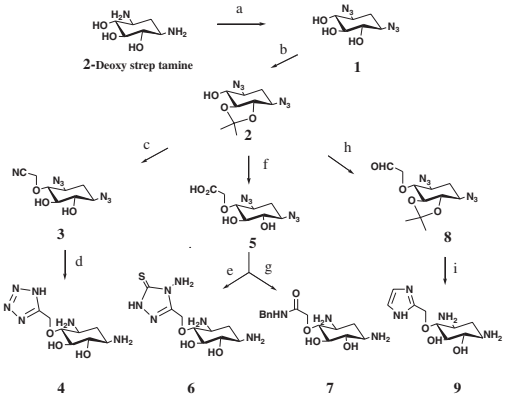
It has been estimated that over one-half of all therapeutic agents consist of heterocyclic compounds. The heterocyclic ring system in many cases comprises the very core of the active moiety or pharmacophore. Therefore, we intend to use 2-deoxystreptamine as a scaffold to synthesize heterocyclic aminoglycosides mimetics as RNA binding motifs.

2-Deoxystreptamine possesses three hydroxy groups and two amino groups, we can simply modify these function groups to prepare the hetercyclic 2-deoxystreptamine libraries. However, we just wanted to connect some interesting heterocyclic moieties with 4-position of 2-deoxystreptamine to make the heterocyclic neamine mimetics.[3]

We already reported the synthesis of N-linked heterocyclic 2-deoxystreptamine derivatives,[4] here we will describe the synthesis of C-linked heterocyclic 2-deoxystreptamine derivatives.

Heterocyclic moieties such as tetrazole,[5] 4-amino-5-mercapto-1,2,4-triazole,[6] and imidazole[7] were considered as interesting heterocyclic pieces, we like to put on 4-position of 2-deoxystreptamine, and the concise synthetic strategy is summarized in Scheme 1.

2-Deoxystreptamine was converted to the diazido derivative **1** in 83% yield by azido transfer reaction with triflic azide.[8] Treatment of compound **1** with 2,2-dimethoxypropane and catalytic amount of camphorsulfonic acid in acetonitrile gave the racemic acetonide **2** in 78% yield. Cyanomethylation of **2** with bromoacetonitrile, followed by removal of the isopropylidene afforded compound **3** in 69% overall yield. Compound **3** was then converted to 5-[(2′-deoxysteptamino)-4′-*O*-ylmethyl]-1*H*-tetrazole (**4**) in 45% yield by reaction with NaN_3 and reduction of the azido groups.[9]



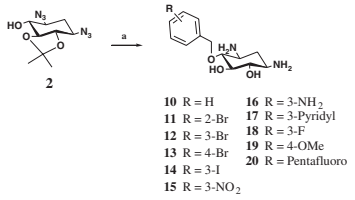
Scheme 1. Reagents and conditions: (a) TfN_3, K_2CO_3, $CH_2Cl_2/MeOH/H_2O$, 10 h; 83%. (b) $CH_3C(OMe)_2CH_3$, CH_3CN, CSA, rt. 78%. (c) i: $BrCH_2CN$, NaH, CH_3CN, 0 °C, 5 h; ii: CH_3CO_2H/H_2O (4:1), 60 °C, 2 h, 69%. (d) i: NaN_3, DMF, 100 °C, 6 h; ii: $Me_3P/THF/H_2O$, 45% for two steps. (f) i: $BrCH_2CO_2Me$, CH_3CN, NaH; ii: CH_3CO_2H/H_2O (4:1), 60 °C, 2 h; iii: $NaOH/MeOH/H_2O$, 68%. (e) i: $CS(NHNH_2)_2$, pyridine, 100 °C, 4 h ii: $Me_3P/THF/H_2O$, 44%. (g) i: $BnNH_2$, DCC, CH_2Cl_2; ii: $Me_3P/THF/H_2O$, 53%. (h) i: CH_2CHCH_2Br, DMF, NaH; ii: OsO_4/NMO, THF/H_2O; iii: $NaIO_4$, THF/H_2O. (i) i: CHOCHO, NH_4Cl, THF/H_2O; ii: CH_3CO_2H/H_2O (4:1), 60 °C, 2 h, iii: $Me_3P/THF/H_2O$, 27%.

Reaction of **2** with methyl chloroacetate followed by acidic hydrolysis of the isopropylidene and saponification of the methyl ester group provided carboxyethyl 2-deoxystreptamine **5** in 68% overall yield. 4-Amino-5-thioxo-3-[(2′-deoxysteptamino)-4′-*O*-ylmethyl]-1,2,4-triazole (**6**) was then obtained in 44% yield from the cyclization of compound **5** with thiocarbohydrazide and reduction of the azido groups.

At meantime, compound **5** was coupled with benzyl amine. After reduction of azido groups, 2-deoxystreptamine benzyl amide derivative **7** was obtained in 53% overall yield.

Synthesis of 2-[(2′-deoxysteptamino)-4′-*O*-ylmethyl]-1*H*-imidazole (**9**) proceeded smoothly as follows. Allylation of compound **2** with allyl bromide, followed by oxidative cleavage of the terminal double bond provided the aldehyde **8**.[10] Condensation of **8** with glyoxal and ammonia chloride followed by deprotection provided compound **9** in 27% overall yield.[11]

Direct benzylation of the compound **2** gave the 4-*O*-(substituted benzyl)-2-deoxystreptamine derivatives. After removal of the isopropylidene and reduction of the azido groups, compounds **10**–**20** were obtained in 35–55% overall yields (Scheme 2).



Scheme 2. Reagents and conditions: (a) i: RCH_2Br, DMF, NaH; ii: CH_3CO_2H/H_2O, 60 °C, 3 h; iii: $Me_3P/THF/H_2O$, 35–55% overall yields.

As heterocyclic neamine mimetics, these final heterocyclic neamine mimetics may not show good activities in the biological screening assay. However, by using ESI-MS RNA binding assay, we still can measure their RNA binding affinities,[12] to provide precise information about their interaction with RNA. This information is useful for us to design and synthesize lead candidate having suitable properties for clinical development.

We have employed ESI-MS RNA binding assay to evaluate the binding affinities of compounds **4**, **6**, **7**, **9**, and **10**–**20** for a 27-*mer* RNA representing the 16S A-site, and the resulting estimated dissociation constants (based on a 1 point K_d determination) are reported in Table 1. During the binding assay, neamine and 2-deoxystreptamine were used as the standard compounds.

Compounds **6** and **9** were found to exhibit higher binding affinities. Based on their structures, design and synthesis of more complex aminoglycoside mimetics for new antibiotics is on the progress.

In conclusion, through an efficient synthetic strategy, we are able to synthesize several different types of 4-heterocyclic 2-deoxystreptamine derivatives as neamine mimetics, which provide an excellent method for the synthesis of heterocyclic carbohydrate libraries. By using ESI-MS RNA binding assay, we are able to find out some useful RNA binding motifs for lead optimization, even they failed in biological screening assay.

Table 1. The dissociation constants of 16*S*-ligand complexes based on gas phase measurements of the ratio of free and bound RNA target

Compounds	Dissociation constants (μM)
4[a]	714.3
6	57.7
7	117.2
9	73.5
10	605.8
11	247.7
12	150.4
13	245.3
14	173.2
15	181.9
16	1328.1
17	339.9
18	203.4
19	240.4
20	166.4
Neamine	24.0
2-deoxystreptamine	600.0

[a]Compound **4** was run at 50 μM, and other compounds were run at 75 μM.

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13 Spectral data for selected compounds. **2**: ^{13}C NMR ($CDCl_3$, 100 MHz) δ 112.7, 79.5, 74.7, 74.0, 62.5, 57.2, 32.0, 26.7, 27.8. **4**: ^1H NMR (D_2O, 400 MHz) δ 8.30 (1H, s), 5.15 (1H, d, $J = 12.4$), 4.93 (1H, d, $J = 12.8$), 3.50 (3H, m), 3.27 (1H, m), 3.12 (1H, td), 2.34 (1H, dt), 1.72 (1H, q, $J = 10.8$); ^{13}C NMR (D_2O, 100 MHz) δ 171, 80.4, 75.6, 72.5, 65.0, 49.7, 49.2, 34.3. **6**: ^1H NMR ($CDCl_3$, 400 MHz) δ 4.93 (1H, d, $J = 13.6$), 3.54–3.38 (3H, m), 3.30 (1H, td, $J = 12.8$, 4.4), 3.16 (1H, td, $J = 13.2$, 4.0), 2.32 (1H, dt, $J = 12.4$, 4.4), 1.70 (1H, q, $J = 12.8$); ^{13}C NMR (D_2O, 100 MHz) δ 176.7, 150.3, 81.2, 75.2, 72.5, 63.8, 50.0, 49.2, 28.2. **7**: ^1H NMR (D_2O, 400 MHz) δ 7.26 (5H, m), 4.35 (1H, d, $J = 2.8$), 4.29 (1H, s), 4.28 (1H, d, $J = 2.8$), 4.24 (1H, s), 3.51–3.35 (4H, m), 3.29 (1H, td, $J = 12.8$, 4.4), 2.31 (1H, dt, $J = 12.0$, 4.0), 1.68 (1H, q, $J = 12.4$); ^{13}C NMR (D_2O, 100 MHz) δ 172.4, 137.9, 129.2, 129.0, 127.4, 81.4, 75.3, 72.5, 70.8, 49.9, 49.0, 42.8, 28.2. **9**: ^1H NMR (D_2O, 400 MHz) δ 7.16 (2H, s), 4.97 (1H, d, $J = 13.6$), 4.91 (1H, d, $J = 14.0$), 3.53–3.23 (4H, m), 3.14 (1H, m), 2.30 (1H, dt, $J = 12.0$, 4.0), 1.68 (1H, q, $J = 12.8$); ^{13}C NMR (D_2O, 100 MHz) δ 120.0, 81.3, 75.3, 72.6, 64.9, 49.9, 49.1, 28.4. **10**: ^1H NMR (D_2O, 400 MHz) δ 7.35 (5H, m), 4.95 (1H, d, $J = 12.6$), 4.69 (1H, d, $J = 12.8$), 3.52–3.44 (3H, m), 3.29–3.17 (2H, m), 2.34 (1H, dt, $J = 12.4$, 4.4), 1.71 (1H, q, $J = 12.4$). **11**: ^1H NMR (D_2O, 400 MHz) δ 7.56 (1H, t), 7.44 (1H, t), 7.31 (1H, q), 7.19 (1H, q), 4.99 (1H, d), 4.72 (1H, d), 3.60-3.45 (3H, m), 3.32–3.18 (2H, m), 2.37 (1H, m), 1.75 (1H, q). **12**: ^1H NMR (D_2O, 400 MHz) δ 7.57 (1H, s), 7.48 (1H, m), 7.34 (1H, m), 7.25 (1H, q), 4.89 (1H, d), 4.69 (1H, d), 3.60–3.48 (3H, m), 3.35–3.22 (2H, m), 2.35 (1H, m), 1.75 (1H, q). **13**: ^1H NMR (D_2O, 400 MHz) δ 7.46 (2H, d, $J = 8.4$), 7.24 (2H, d, $J = 8.4$), 4.83 (1H, d, $J = 11.2$), 4.62 (1H, d, $J = 11.2$), 3.50–3.41 (3H, m), 3.29–3.15 (2H, m), 2.34 (1H, dt, $J = 12.4$, 4.4), 1.71 (1H, q, $J = 12.0$). **14**: ^1H NMR (D_2O, 400 MHz) δ 7.70 (1H, s), 7.58 (1H, d, $J = 7.6$), 7.29 (1H, d, $J = 7.2$), 7.03 (1H, t, $J = 7.2$), 4.78 (1H, d, $J = 11.2$), 3.45 (3H, m), 3.29–3.15 (2H, m), 2.31 (1H, dt), 1.71 (1H, q, $J = 12.8$).

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