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Synthesis of neamine libraries for RNA recognition using solution phase chemistry

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Abstract

Selective protection of the 6′-amino group of neamine allows the preparation of aminoglycoside libraries by reductive amination and Ugi multicomponent coupling.

Keywords: antibiotics; amino sugars; combinatorial chemistry; Ugi–Passerini reactions.

Targeting of non-ribosomal RNAs by small, non-protein molecules is an attractive option for treatment of a number of disease states.[1] However, selective recognition of RNAs of therapeutic interest is a challenging problem. There are relatively few examples of small molecules which can bind RNAs with any selectivity.[2,3] There is a need both to increase the structural diversity of the molecules which bind selectively, and to develop our understanding of the structural basis for the recognition process.[4] A particularly interesting feature of such recognition is the highly variable tertiary structures that RNAs can adopt. The discovery that the aminoglycoside antibiotic neomycin B **1** binds to a number of important RNAs,[2] including the rev response element (RRE) of the HIV-1 RNA, has stimulated a number of studies in this area. Neomycin itself is highly toxic, difficult to manipulate, and is prone to degradation by hydrolysis of the ribose glycosyl bond. Hence, both the previously published studies[5–7] and this work concentrate on the preparation of libraries based on smaller scaffolds, either 2-deoxystreptamine[6] or neamine **2**[7] (Scheme 1). Even so, the preparation of aminoglycoside libraries is a demanding undertaking. Previously, it has been shown that supported libraries of neamine derivatives can be prepared by functionalisation of the 5-hydroxyl group.[7] In contrast, the use of amino functionality to construct neamine libraries has not been reported. The heteroatom chosen for derivatisation is important since the protecting group chemistry should be as simple as possible. We wish to report a revised selective *N*-protection regime for neamine and the preparation of libraries using the 6′-amino group.

Neamine free base can be prepared from commercial neomycin[7,8] by acidic methanolysis (Scheme 1) followed by treatment with Amberlite IRA-400 (OH^- form). The known methods for the selective *N*-

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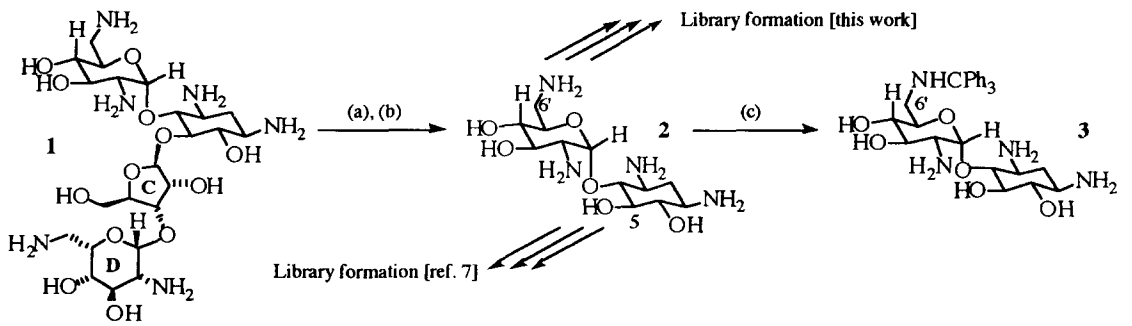
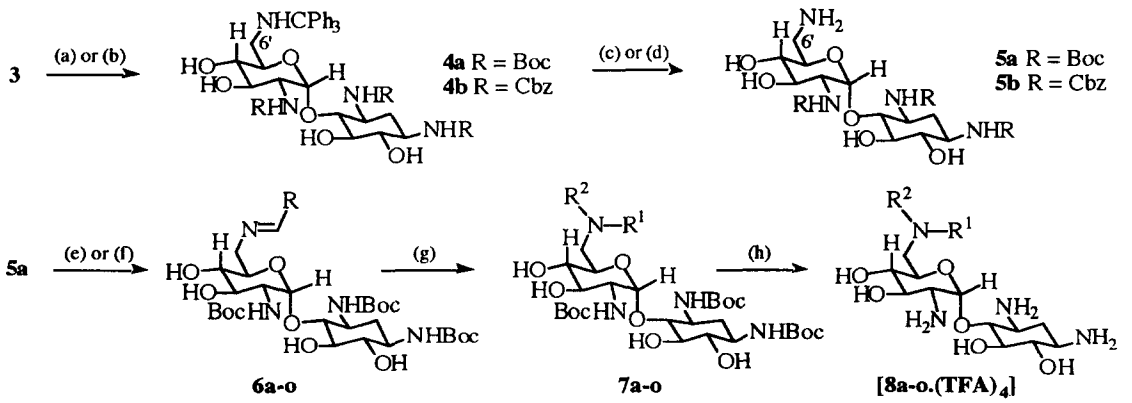


Scheme 1. Reagents and conditions: (a) MeOH, HCl, reflux, 16 h, 67%. (b) Amberlite IRA-400 (OH^-), MeOH:water 1:1 v:v, 0.5 h. (c) 1.5 equiv. Ph_3CCl, Et_3N, MeOH:dioxane:water 1:1:1 v:v:v, 20°C, 18 h, 52%

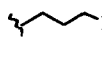
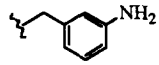
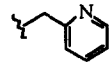
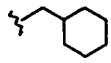
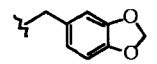
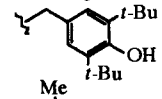
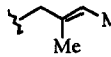
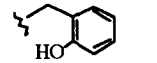
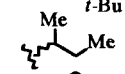
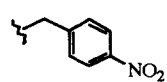
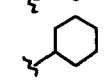
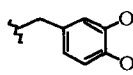
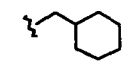
protection of neamine proved to be unsuitable. The use of transition metal ions, e.g. Cu^{2+} or Ni^{2+}, to protect the 1,2-amino alcohol motifs in situ was ineffective. Equally, the reagent Cbz-NBD, used previously to protect the 6′-amino group of neamine,[8] was unreactive towards the aminoglycoside in our hands. Screening of a variety of protecting group regimes, e.g. Cbz, Boc, Bn, and PMB, resulted in the adoption of tritylation as the favoured method (Scheme 1). Such a sterically demanding protecting group cleanly discriminated between the amino groups based on their attachment to methylene and methine carbon atoms. The monotritylated product **3** was prepared in 52% yield after chromatography. The moderate yield reflects the difficulty in avoiding adventitious hydrolysis of the trityl chloride under the necessary aqueous conditions rather than any lack of *N*-selectivity. Further orthogonal *N*-protection (Scheme 2) was achieved by reaction with $(Boc)_2O$ (giving **4a** in 63% yield) or Cbz-Cl (giving **4b** in 45% yield) under standard conditions. The Boc protection was superior in terms of practicality although one of the possible tetra-Boc compounds, i.e. containing a $-N(Boc)_2$ residue, was isolated as a byproduct. However, this impurity had no effect on the subsequent chemistry and the mixture could be carried forward without the need for a tedious separation. Detritylation of **4b** (AcOH:EtOH 4:1, 4 h, 20°C) was readily accomplished in 63% yield, but curiously, somewhat more forcing conditions (48 h, 50°C) were required to deprotect **4a**, giving **5a** in 90% yield. These protecting group manipulations allow the preparation of significant quantities of a neamine scaffold suitable for incorporation into libraries.



Scheme 2. Reagents and conditions: (a) 4 equiv. $(Boc)_2O$, 3 equiv. NaOH, dioxane:water 1:1 v:v, 20°C, 18 h, 63% **4a**. (b) CbzCl, satd Na_2CO_3, acetone:toluene 7:1 v:v, 0°C→20°C, 18 h, 45% **4b**. (c) **4a** AcOH:EtOH 4:1 v:v, 50°C, 48 h, 90% **5a**. (d) **4b** AcOH:EtOH 4:1 v:v, 20°C, 4 h, 63% **5b**. (e) 1.5 equiv. RCHO, DMF, 3Å MS, 70°C, 18 h. (f) 1.5 equiv. RCHO, Et_3N (1.5 equiv.), MeOH, 3Å MS, 40°C, 18 h. (g) $NaBH_4$ on Amberlite IRA-400, MeOH, 40°C, 3 h. (h) TFA, 20°C, 0.5 h

Replacement of the 6′-amino group of neomycin B by hydroxyl gives paromomycin, an antibiotic

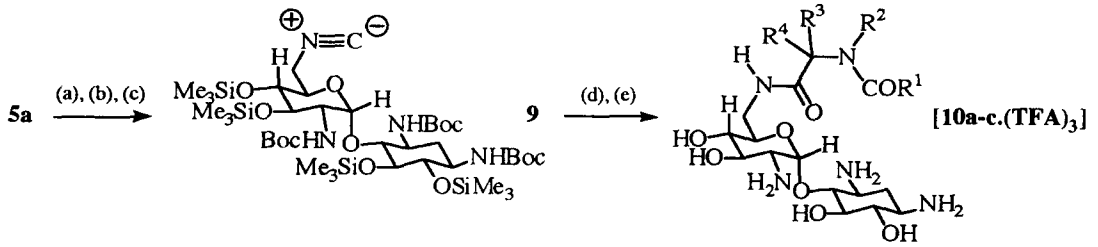
Table 1
Reductive amination of **5a** giving **8**[a,b]

Entry	R^1		Entry	R^1		Entry	R^1	
a		36%	**f**		39%	**k**		34%
b		27%	**g**		46%	**l**		51%
c		26%	**h**		29%	**m**		41%[c]
d		42%	**i**		60%	**n**		26%
e		43%	**j**		67%	**o**		R^2=  46%

a) For the conditions see Scheme 2. b) R^2 = H unless stated otherwise. c) Compound **8m** is a 1:1 mixture of diastereoisomers.

known to bind less well to the RRE.[2,9] Hence, significant changes in the binding selectivity might be expected by additional substituents at the 6′-position. Furthermore, such libraries are complementary to those previously reported[7] and explore distinct regions of the binding domain. Given the likely importance of electrostatic interations involving the 6′-amino functionality on the binding, we sought to preserve the basic site but modify it by alkylation (Scheme 2). Direct reductive alkylation of **5a** free base under standard conditions, e.g. catalytic *p*TSA, 3Å MS, dry MeOH, was unsuccessful. However, the intermediate imines were formed by heating the acetate salt of **5a** with an aldehyde (1.5 equiv.) at 70°C in DMF.[10] Alternatively, stirring the acetate salt of **5a** with an aldehyde in dry MeOH (40°C) buffered by Et_3N also gave the imines in good yields (typically around 60% isolated yield). Subsequent reduction ($NaBH_4$ on Amberlite IRA-400) could be performed under either set of conditions without isolation of the intermediates (Scheme 2, Table 1). The products **7** were isolated by filtration and evaporation. Deprotection (**7**→**8**) of the reduction products was accomplished by treatment with TFA and the products were purified by evaporation and chromatography using SiO_2. The compounds illustrated in Table 1 have been taken through the sequence **5**→**6**→**7**→**8** with very clean conversions based on TLC analysis. The variable isolated yields reflect the fact that no attempt has been made to optimise the purifications, which were performed on a very small scale. Nonetheless, these examples demonstrate the range of aldehydes, e.g. aliphatic, α-branched, aryl, and α,β-unsaturated, that can be used. At present, we are exploring the incorporation of more functionalised carbonyl compounds. Although direct reaction of **5a** with dialkyl ketones was slower and required the use of DMF at 70°C, the imines were still formed with high conversions and two examples are illustrated (Table 1, entries **m** and **n**). It is also possible to doubly *N*-alkylate by sequential reductive amination. This methodology is exemplified by the conversion of **7b** into **7o** (Table 1). However, alkylation of **7k** (and related benzylic compounds) by aromatic aldehydes has failed so far. Nonetheless, this methodology provides access to large numbers of mono- and dialkylated neamine derivatives that preserve the basic site at C-6′.

The selective protection chemistry described herein allows further functionalisation of the neamine scaffold. In order to prepare larger compound libraries, we have investigated Ugi coupling chemistry[11] of the known isonitrile **9**[8] (Scheme 3). The use of an isonitrile derivative of neamine overcomes the problems caused by the limited commercial availabilty of isonitriles in general and therefore, should allow for greater library diversity.



Scheme 3. Reagents and conditions: (a) $(Me_3Si)_2NH$, Me_3SiCl, pyridine, 20°C, 16 h, 81%. (b) *p*-nitrophenylformate, THF, 50°C, 16 h, 64%. (c) 1.5 equiv. TsCl, pyridine, 20°C, 16 h, 56%. (d) 3 equiv. R^1CO_2H, R^2NH_2, R^3CHO (R^4=H), DCM, 20°C, 48 h. (e) TFA, 20°C, 0.5 h

Table 2
Ugi coupling reactions of **9** giving **10**[a,b]

Entry	R^1	R^2	R^3	Yield
a	Me	*i*-Pr	Me	ND[c]
b	Me	*i*-Pr	Ph	75%
c	Me	Ph	3-pyridyl	80%

a) For the conditions see Scheme 3. b) R^4 = H (c) ND = yield not determined.

Preparation of **9** was accomplished using a modification of the literature route (Scheme 3). *O*-Silylation (HMDS, TMSCl, pyridine) of the intermediate formamide was necessary to improve the solubility prior to dehydration and Ugi reaction. Dehydration using modified conditions (TsCl, pyridine, 20°C, 16 h) afforded the fully protected isonitrile **9** in 56% yield. This isonitrile underwent Ugi reactions (DCM, 20°C, 48 h) with a variety of aldehydes, amines, and acetic acid with good conversions. The co-reagents were used in excess to ensure complete conversion to the coupled products. The products were globally deprotected in situ using TFA and the Ugi products **10** isolated as their TFA salts by evaporation of the volatiles. Illustrative examples are provided in Table 2. Purification could be accomplished prior to deprotection with TFA but this is unnecessary for biological screening. Overall, this chemistry provides a straightforward but flexible approach to constructing aminoglycoside libraries since the connectivity of the coupled products can be varied by either using amine **5a** or isonitrile **9** in multicomponent coupling reactions. Combining **5a** with **9** should allow the preparation of 'multivalent' analogues[12] in a library format.

In summary, we have shown that simple selective tritylation of neamine can be used to allow access to neamine libraries based on solution phase reductive amination chemistry and Ugi coupling chemistry. We are exploring other solution phase chemistry based on this selective protection regime. The compounds are currently undergoing screening for binding to a number of RNAs of therapeutic interest and the results will be reported in due course. Moreover, this simple protection chemistry allows easy access to solid supported neamine derivatives. Our current efforts are directed at perfecting the solid supported chemistry in order to construct larger and more diverse aminoglycoside libraries and minimise the problems associated with compound isolation.

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