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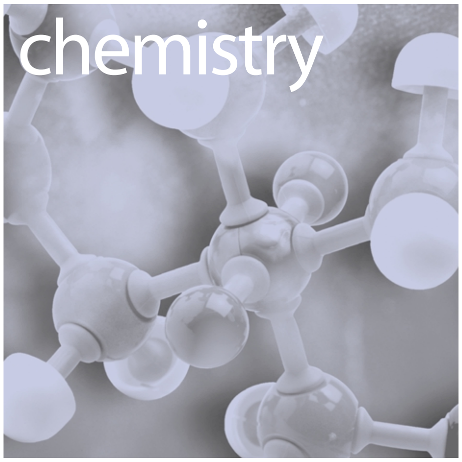
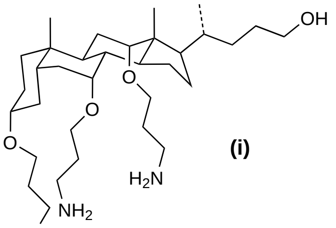


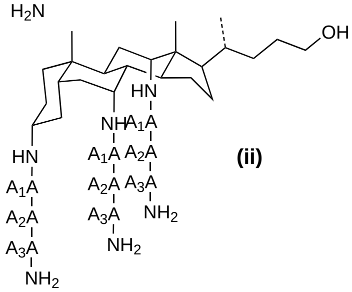
MOLECULES

Peptide–cationic steroid antibiotic conjugates

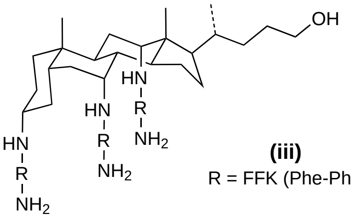
Cationic steroid antibiotics were developed to mimic the antibacterial behaviour of endogenous peptide antibiotics, including selective association of the antibiotics with, and disruption of, bacterial membranes. This association and disruption behaviour results in rapid bacterial activity with a minimal potential for causing the emergence of resistance. Many of these antibiotics adopt cationic, facially amphiphilic conformations [such as **(i)**]. The antibacterial properties of these antibiotics appears to highly dependant upon these conformations. Membrane selectivity is derived primarily from ionic recognition of negatively charged bacterial membranes. Bacterial membrane components, however, present more than just anionic groups, so it could be expected that additional interactions might increase the affinity of membrane-active antibiotics for bacterial membranes and hence increase antibacterial efficacy. As a result of these findings and the arising hypothesis outlined above, work has been directed toward increasing the functionality presented by cationic steroid antibiotics [1].



(i)



(ii)



(iii)
R = FFK (Phe-Phe-Lys)

The cationic steroid antibiotics are relatively rigid structures displaying significant preorganization, so any functional groups intended to interact with bacterial membrane components should be situated on the polar face. In this article, a library of 216 peptide cationic steroid antibiotic conjugates were prepared with the general structure outlined in **(ii)**. Following preparation of this library, compounds were screened for activity against Gram-negative (*Escherichia coli* (ATCC 25922)) and Gram-positive (*Staphylococcus aureus* (ATCC 25923)) bacteria using a micro-broth dilution method. From this effort, sequences of tripeptides yielding cationic steroid antibiotic–peptide conjugates with good antibacterial properties were found. One of the most potent compounds discovered was **(iii)** which possessed an MIC of 8 μg/mL against *S. aureus* and *E. coli*. This work is of interest as it rapidly identified key requirements for peptide-containing cationic steroid antibiotics and discovered compounds that appear to offer an improvement in antibacterial activity. Further work to expand the scope of amino acids incorporated within the libraries synthesised is warranted in order to improve antibacterial activities against Gram-negative and Gram-positive bacteria.

Stabilization of oligonucleotide complexes

Selective molecular recognition between synthetic oligonucleotide ligands and nucleic acid targets plays a pivotal role in most aspects of molecular biology and biotechnology. Natural DNA or RNA oligonucleotides, either identified *via in vitro* selection or through rational design will usually generates lead compounds. Improving the oligonucleotide's drug properties, such as affinity, nuclease resistance and membrane permeability, often requires chemical modifications. Such chemical modification can prove difficult, especially for those molecules intended to bind to RNA targets. Recent work has been directed towards the identification of covalently appended small molecules that stabilize nucleic acid complexes through the use of dynamic combinatorial chemistry [2].

The emerging field of dynamic combinatorial chemistry involves the use of reversible reactions, generating an equilibrating mixture of molecules, that is a dynamic combinatorial library (DCL). The composition of this DCL is able to respond to molecular-recognition events resulting from the addition of a target of interest. The preferential binding of one member of the DCL to the target induces a shift in the equilibrium towards the formation of that particular compound. Whereas traditional combinatorial chemistry library synthesis and screening are two separate, sequential, processes, dynamic combinatorial

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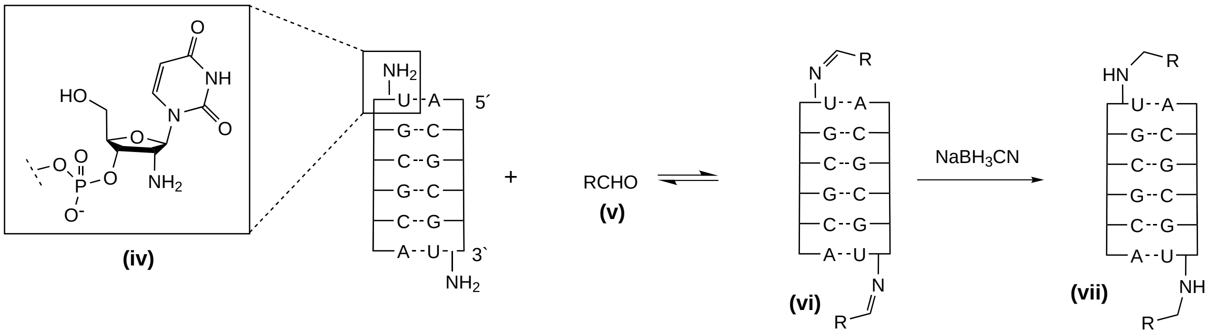

chemistry offers *in situ* screening of the combinatorial library by comparing its composition in the presence and absence of the target.

Bugaut *et al.* [2] use this concept with an oligonucleotide ligand bearing a reactive amino group (iv) which is reacted reversibly with a set of aldehydes **(v)** in aqueous media. The resulting mixture of imines **(iv)** at equilibrium is responsive to its environment. The addition of a nucleic acid target that develops interactions with imine products should promote the formation of the strongest binders. Subsequently, the mixture of interconverting imine species is reduced with sodium cyanoborohydride to form chemically stable amines **(vii)**, facilitating isolation and analysis of the final mixture. This concept was used to provide for the first successful use of dynamic combinatorial chemistry for the rapid identification of a non-nucleic acid residue appended to an oligonucleotide ligand that stabilizes the complex formed with its nucleic acid target. This was achieved with a DNA duplex and with a tertiary-structured RNA–RNA complex. Thus dynamic combinatorial chemistry represents a powerful methodology for optimization of nucleic acid ligands. Further work is being undertaken to expand the set of aldehydes and 2´-amino-2´-deoxynucleotides present in the ligands used.

1 Ding, B. *et al.* (2004) Synthesis and characterization of peptide-cationic steroid antibiotic conjugates. *Org. Lett.* 6, 3433–3436

2 Bugaut, A. *et al.* (2004) Use of dynamic combinatorial chemistry for the identification of covalently appended residues that stabilize oligonucleotide complexes. Angew. *Chem., Int. Ed.* 43, 3144–3147

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