Synthesis of substituted Aza Crown Ethers as Metal- and Amino Acid Binding Sites in DNA-conjugates

Stefan Vogel and Jesper Wengel

Nucleic Acid Center
Department of Chemistry, University of Southern Denmark, Campusvej 55
DK-5230 Odense M, Denmark

Introduction:
Modified and functionalised oligonucleotides have been synthesized and used as molecular tools. Beside the many existing modifications a very limited number of binding sites for organic molecules has been inserted into oligonucleotides. Special interest in terms of host-guest chemistry have functionalised crown ethers. Their selectivity for metal cations of different sizes and complexation of organic molecules such as amines and amino acids is a starting point for promising applications. Especially triaza crown ethers show a remarkable affinity for protonated primary amines (See Table 1). A number of appropriate modified oxo- and aza crown ethers has been synthesized and functionalised to be compatible with automated synthesis using the phosphoramidite approach.

Synthesis of asymmetric substituted triaza crown ethers:
Commercially available 1 was treated with benzoic anhydride and subsequently mesylated to give building block 2. Treatment of 2 with primary amines afforded diamides 3a, 3b. Reduction of the diamides to the corresponding amines lead to suitable intermediates for a macrocyclization reaction. Treatment of the diaminos with dethenehydroxylation gave the macrocycles 4a and 4b. Debenzylation and reductive amination gave macrocycles 5a and 5b. Phosphitylation of 5a afforded compound 6. DMT- protection of the primary hydroxyl group of 5b followed by phosphorylation gave compound 7.

Triaza crown ether macrocycles:
In general, crown ether macrocycles bind alkali cations appreciable stronger than RNH₂⁺ groups, whereas the opposite selectivity would be desirable for the design of receptor molecules aimed at the complexation of organic substrates. This is achieved with the mode of complexation in triaza crown ethers (See Fig. 1).

Table 1. Stability constants K for the cation binding by crown ether macrocycles

<table>
<thead>
<tr>
<th>Macrocycles</th>
<th>K⁺</th>
<th>CH₃-NH₃⁺</th>
<th>Ph-(CH₂)₂-NH₃⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>18-crown-6</td>
<td>170000</td>
<td>2100</td>
<td>1500</td>
</tr>
<tr>
<td>18-crown-6N₂</td>
<td>12500</td>
<td>2200</td>
<td>2000</td>
</tr>
<tr>
<td>18-crown-6N₂</td>
<td>60000</td>
<td>66000</td>
<td>50000</td>
</tr>
</tbody>
</table>

Figure 1. Illustration of the complexation of ammonium cations by triaza crown ethers through a network of hydrogen bonds.

Synthesis of oxo crown ethers:
Commercially available 8 is converted to diol 9 by Sharpless dihydroxylation followed by protection of the primary hydroxyl group with DMTCl. Compound 10 is finally converted to the corresponding phosphoramidite 11.

Scheme 2. a) AD-Mix-alpha, H₂O/ t-BuOH, 36°C, 80%; b) DMTCl, pyridine, r.t., 60%; c) 2-cyanoethyl- N,N-diisopropylphosphoramidochloridite, CH₂Cl₂, r.t., 70%.

Discussion:
A family of asymmetric substituted triaza crown ethers has been synthesized based on the Krakowiak building block method. ¹ The synthetic route is short and high yielding with high degree of flexibility regarding substitution pattern on all N-atoms. A number of triaza crown ethers are available which are suitable for incorporation into oligonucleotides (ON) using automated synthesis based on the phosphoramidite approach. The synthesis of functionalised oxo crown ether amidites is achieved starting from commercially available 4-vinylbenzo-18-crown-6 in only 3 steps with a Sharpless dihydroxylation as the key step. The DMT-protected oxo crown ether amidite 11 can be used for automated ON synthesis. However, preliminary attempts to use crown ether amidites for automated synthesis of ON were not successful.

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References: