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Synthesis and DNA binding ability of cyclam–amino acid conjugates

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Abstract—*N*-Substituted cyclam–amino acid conjugates have been synthesised both in solution and on the solid phase. The DNA binding affinity of these species has been studied: the nature of the amino acid strongly influences the change in melting temperature suggesting that simple cyclam–peptide conjugates could interact with DNA in a highly selective manner.

The interaction of small molecules[1] and metal nucleases[2] with DNA has attracted much attention in chemistry, biology and medicine. In this context, tetraazamacrocycles are of interest due to their ability to form stable metal complexes which are able to hydrolytically cleave DNA.[3] There is even preliminary evidence that such ligands are able to cleave DNA in the absence of metal ions.[4] The ligands also have potential applications as anti-tumour and anti-HIV agents.[5]

Nature exquisitely controls the site-selective cleavage of DNA with metal nucleases by employing peptide-based interaction domains, for example, in restriction enzymes.[6] The introduction of such peptidic side chains into azamacrocycles could permit sequence selectivity in their interaction with DNA, as well as allowing the fine-tuning of other parameters such as lipophilicity and bioavailability, leading to sequence-selective small molecule nucleases or binders, of which there are still few examples.[7] Such an approach could generate a new class of molecular therapeutics based on selective interactions with problematic DNA sequences, for example, the TT(N5)AA consensus sequence that binds to *Stat-5*, a transcription factor implicated in prostate and breast cancers,[8] or the G-rich sequence of telomeres, associated with 'immortalised' cancer cell lines.[9]

Among the various azamacrocycles, cyclam (1,4,8,11-tetraazacyclotetradecane) and its *N*-substituted derivatives have attracted much attention due to their wide range of applications in medicine.[10] Peptide nucleic acids with a terminal cyclam have been synthesised and their interactions with DNA have been studied.[11] We sought to apply our recent synthetic experiences in the synthesis of cyclam analogues functionalised with biotin[12] to the synthesis of cyclam–amino acid conjugates, although there are other literature examples of the synthesis of cyclam–oligopeptide conjugates both in solution and on solid phase supports, primarily for radiolabelling applications.[13] Whilst there have been syntheses of cyclam–amino acid conjugates in which the linking bond is the amide arising from a cyclam nitrogen and the amino acid carboxylic acid,[14] we, and others, have found the metal complexes of such species to be unstable with respect to hydrolysis.[15] As our ultimate goal of this research is the synthesis of functionalised azamacrocyclic complexes for the sequence-selective cleavage of DNA that will rely on the presence of the peptide chain, such behaviour would preclude the use of this linker. As the first step, we report here the synthesis of several novel cyclam–amino acid conjugates and their interaction with calf thymus (CT) DNA.

Synthesis of cyclam–amino acid conjugates. *Solution phase synthesis*. Commercially available cyclam **1** was treated with di-*tert*-butyldicarbonate to give the tri-Boc derivative **2** using standard literature methodology (Scheme 1).[12,16] This was further coupled with ethyl bromoacetate to give **3**, which on hydrolysis under

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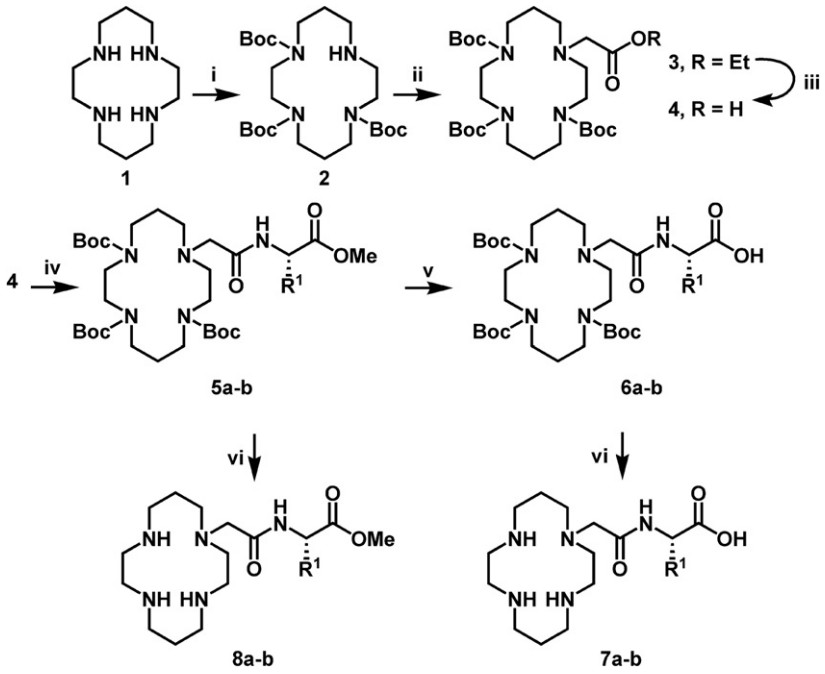
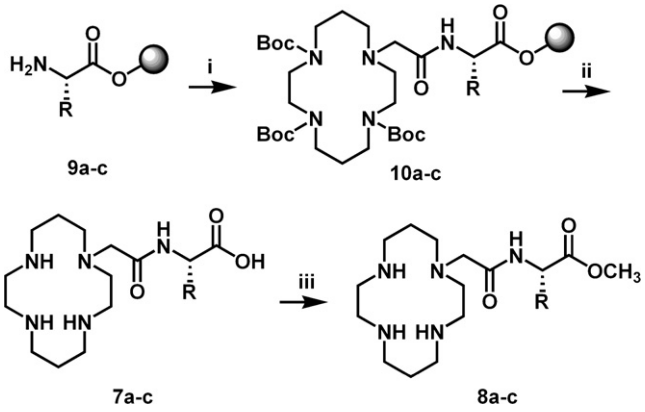


Scheme 1. Reagents and conditions: (a) R^1 = Me; (b) R^1 = Bn. (i) $(Boc)_2O$, Et_3N, DCM, −15 °C to rt, 12 h, 53%; (ii) $BrCH_2CO_2Et$, Na_2CO_3, CH_3CN, 85 °C, 16 h, 97%; (iii) NaOH, MeOH, rt, 5 h, 68%; (iv) $NH_2CHR^1CO_2Me$, DCC, DMAP, DMF, DCM, rt, 12 h, 60–75%; (v) NaOH, MeOH, rt, 6 h, 78–85%; (vi) TFA, DCM, rt, 12 h, 80–85%.

standard conditions gave acid **4**. This acid was coupled with alanine or phenylalanine amino acid esters to give the novel compounds **5a**–**b**. Direct saponification of the methyl esters in **5** was readily achieved to provide the acid intermediates **6a**–**b** from which the free macrocycles **7a**–**b**, respectively, could be liberated using TFA. Analogous esters **8** were obtained by direct Boc deprotection of **5** using TFA. This sequence, though requiring protection and deprotection steps, is experimentally quite simple and proceeds in an overall yield of approximately 15% for the six steps. However, one of the low yielding steps is the peptide coupling. In our previous work on a related reaction we discovered that HATU gave yields of 80% or more for this kind of coupling reaction,[12] but if we were to iterate this coupling step in the synthesis of cyclam ligands functionalised with oligopeptide arms, the final yield would become unacceptably low. We consequently examined an alternative solid phase synthetic approach to these molecules.

Solid phase synthesis. Cyclam–amino acid conjugates were synthesised on the solid phase by employing standard Fmoc methods using chlorotrityl resin. The resin was loaded with an Fmoc protected amino acid, which on deprotection gave the supported amine **9**. The amine could then be coupled to acid **4** to give the supported conjugate **10**. The free acid **7** could then be liberated from the resin on treatment with TFA and converted to their methyl esters **8** in solution on treatment with H_2SO_4 in MeOH (Scheme 2). Using this method we were also able to synthesise the serine analogues **7c** and **8c**; these had given poor yields when synthesised by the solution phase approach.



Scheme 2. Reagents and conditions: (a) R = Me; (b) R = Bn; (c) R = CH_2OBn. (i) Compound **4**, DIPEA, HBTU, DCM, 12 h; (ii) TFA, DCM, 10 h; (iii) H_2SO_4, MeOH, 5 h.

The solid phase synthesis of these compounds presents considerable advantages over the solution phase as we were able to prepare these compounds with high purity and in higher yields than the corresponding solution phase methods (Table 1, yields based on comparable sequences for the two routes). The solid phase method provided a significantly more expedient route to these compounds than the corresponding solution phase approach. All compounds synthesised by the solid phase approach were consistently around 90% pure as judged by HPLC analysis of the isolated material with no further purification.[17]

DNA binding experiments. The DNA-binding ability of these new cyclam–amino acid conjugates was investi-

Table 1. Comparative yields for solution versus solid phase synthesis of cyclam–amino acid conjugates

Compound	% Yield in solution phase[a]	% Yield on solid phase[b]
7a	34	90
8a	51	90
7b	39	85
8b	52	82
7c	—	84
8c	—	78

[a] Yields based on yield of final product from **4**.
[b] Yields based on steps between **9** and isolated product after removal from resin.

gated by thermal denaturation studies using calf thymus (CT) DNA (Fig. 1). Melting studies show that these compounds stabilize the thermal helix coil or melting stabilization (ΔT_m) for the CT-DNA duplex at pH 7.0. The compound/DNA molar ratios measured were 1:3 and 1:5. For the acids (**7a**–**c**) the change in melting temperature (ΔT_m) is variable, showing that the extent of stabilisation is governed by the amino acid employed. The esters **8a**–**c** in contrast exhibit far less stabilisation of the helix. These results are particularly pleasing when it is observed that cyclam itself does not appreciably stabilise the helix, and the tri-Boc acid **4** does not stabilise the helix at all. Further the magnitude of the increase in melting temperature for compound **7c** is approximately 8 °C, which is nearly as large as that observed for ethidium bromide, our reference compound used for these experiments.[18] It is particularly surprising that a relatively large increase in melting temperature can be generated with the addition of only one amino acid. This is a significant result, and suggests it might be possible to tune the stabilisation of the DNA helix with polypeptides on more elaborate cyclam-based structures. The mode of interaction with DNA was electrostatic in nature, confirmed by a gradual lowering of the ΔT_m when the melting temperatures were measured after the addition of increasing quantities of 1 M NaCl solution to the original buffer.

In conclusion, we have demonstrated the synthesis of novel cyclam–amino acid conjugates in both solution

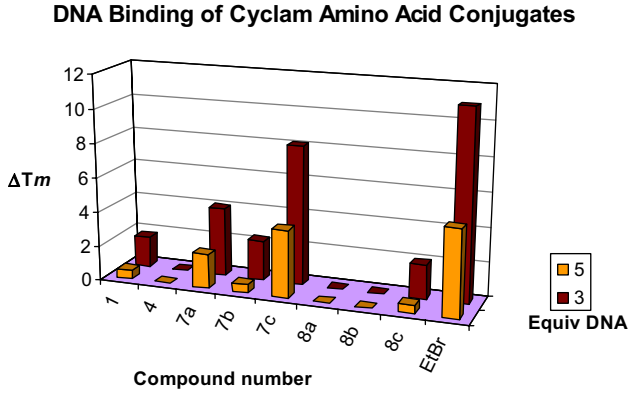


Figure 1. Changes in calf thymus DNA melting temperatures upon addition of varying molar ratios of cyclam, cyclam–amino acid conjugates and ethidium bromide (errors in measurements approximately ±0.5 °C).

and solid phase. The solid phase approach has a number of distinct advantages in terms of yield and operational simplicity, and will be a powerful method for the synthesis of more elaborate cyclam–peptide conjugates. These compounds have been further studied for their ability to bind to DNA. It was found that the nature of the amino acid employed greatly affected the increase in melting temperature. We observed a large increase in melting temperature for the cyclam attached to *O*-benzyl-protected serine. It is possible that the stabilisation arises from the intercalation of the benzyl group with the DNA bases. However, the stabilisation observed with the phenylalanine analogue was considerably lower in magnitude. It is possible that the protected oxygen of the serine is involved in a favourable hydrogen-bonding interaction with the DNA. The deprotected form of **7c** is therefore of further interest. The assessment of the ability of the corresponding metal complexes of these derivatives to bind DNA and cleave simple phosphate esters is currently underway.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2008.03.045.

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