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Solid-Phase Synthesis of Symmetrical 3,6-Bispeptide–Acridone Conjugates

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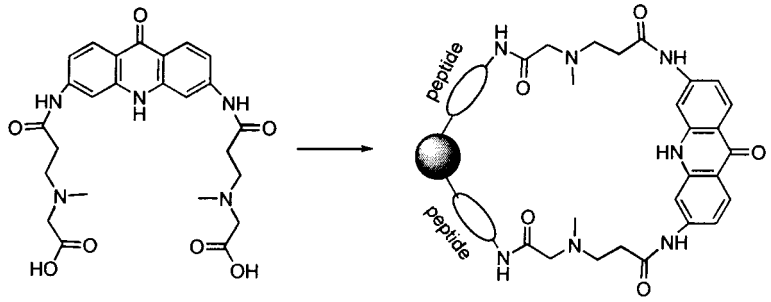
ABSTRACT



A novel high-yielding method for the solid-phase synthesis of 3,6-bispeptide–acridone conjugates is reported. It involves initial coupling of bifunctionalized acridone to a resin-bound peptide followed by an on-bead site–site reaction to couple the second peptide. This method leads to clean symmetrical bispeptide derivatives and appears to be general. This strategy will enable the generation of a library of 3,6-bispeptide–acridones to be screened for selective binding to telomeric G-quadruplex DNA.

The design of small molecules that bind telomeric DNA with high affinity and specificity is a potential approach for interfering with the regulation of telomeres of tumor cells in culture.[1] This concept has been stimulated by recent studies that have demonstrated that stabilization of the telomeric DNA folded into four-stranded guanine-quadruplex (G4) structures with small molecules is an effective way to inhibit telomerase.[2] It has also been shown that ligands that stabilize G4 can elicit various other biological effects that include inhibition of specific helicases by preventing G4 unwinding[3] and influence of transcriptional control by the c-*myc* oncogene promoter.[4] A number of heterocyclic synthetic molecules have been identified as G4 binders that also inhibit telomerase activity,[5] which include a series of cationic porphyrins,[6] perylenes,[7] amidoanthraquinones,[8] and deriva-

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tives based on an acridone scaffold such as 3,6-diamidoacridones[9] or 3,6-diamido-9-anilinoacridines.[10] In particular, a series of 3,6,9-trisubstituted acridines have proven to be particularly selective at targeting G-quadruplexes. Acridine **1** (Figure 1) was recently shown by Neidle and co-workers

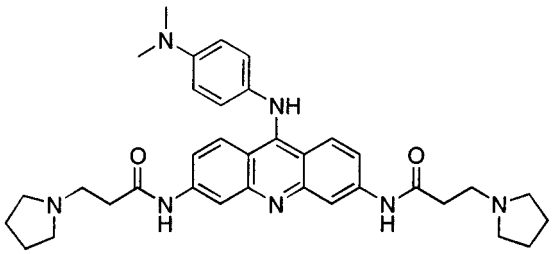

Figure 1. 3,6,9-Trisubstituted acridine **1** as a potent and selective G-4 binder.

to bind G-quadruplex DNA (K_d = 100 nM) about 40 times more tightly than duplex DNA (K_d = 4 μM)[10] and exhibit antitumor activity.[11]

A working hypothesis for the way heterocycles bind G-quadruplexes has been presented in molecular modeling studies.[10,12] In particular, one study[10] suggests that acridine **1** is stabilized on the terminal G quartet by $\pi-\pi$ interactions. The two-substituent amidoalkylamino chains are orientated in the two widest grooves, with the two terminal pyrrolidine rings involved into hydrophobic interactions with the sides of these grooves. This supports the theory that peptides on positions 3 and 6 of the acridone scaffold should be directed into two different grooves by analogy with the pyrrolodine rings of compound **1**. This general binding model has been further supported by a recent X-ray structure of a small-molecule−G-quadruplex binary complex.[13]

Starting from the 3,6-disubstituted acridone core, we have demonstrated an efficient method for the solid-phase synthesis of a library of symmetrical 3,6-bispeptide−acridone conjugates. The molecular design is intended to exploit the π interaction of the heterocyclic core to orientate both peptides into two different grooves of the G4 akin to the two pyrrolidine rings of the G4 binding compound **1**. Oriented peptidic side chains offer the potential for stronger, more selective interaction with the grooves of the quadruplex and thus more potent inhibitors.

This strategy of coupling distinct recognition elements to generate a ligand for specific recognition of biological targets is similar to approaches employed by others[14,15] to inhibit protein−protein interactions. To establish synthetic methodology and generate some first-generation compounds, we have employed tetra- and hexapeptides that have been selected from a combinatorial library and found to bind weakly but specifically to G4,[16] in order to obtain bispeptide−acridone conjugates.

A route to monopeptide−acridine conjugates has been reported using amidic coupling between a resin-bound peptide and the *N*-hydroxysuccinimide-activated ester of an acridine 4-carboxylic acid.[17−18] The methodology we describe here exploits on-bead site−site reactivity. Site−site interactions were discovered quite soon after the invention of solid-phase synthesis.[19] More recently, Yan et al.[20] have shown that site−site interactions occur in 1% DVB cross-linked polystyrene resin of 0.9 mmol/g load capacity due to the highly dynamic nature of the polymer matrix. Site−site reactivity will be dependent on both the chemical reactivity and the proximity of the reacting groups. While examples of site−site interactions in solid-phase organic synthesis are generally reported as a problem to be minimized, the strategy described here deliberately exploits this type of reactivity to generate bis-substituted heterocycles.

A few strategies have already been described in the literature for the solid-phase synthesis of homodimeric compounds, but involving either a bifunctional scaffold bound to the resin (Figure 2a)[21] or "intra-site" reactions for

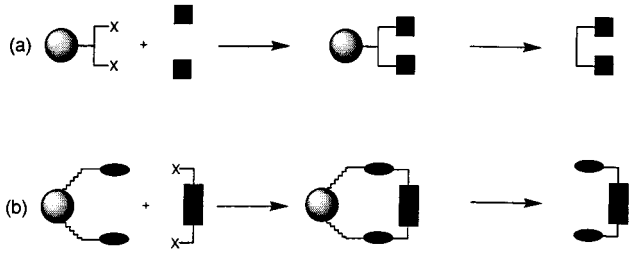

Figure 2. Two strategies for the solid-phase synthesis of homodimeric molecules by using either a (a) bifunctional scaffold bound to the resin[21] or (b) bifunctional ligand.

the dimerization of two reactive identical monomers.[22] The strategy presented here exploits a site−site reaction between

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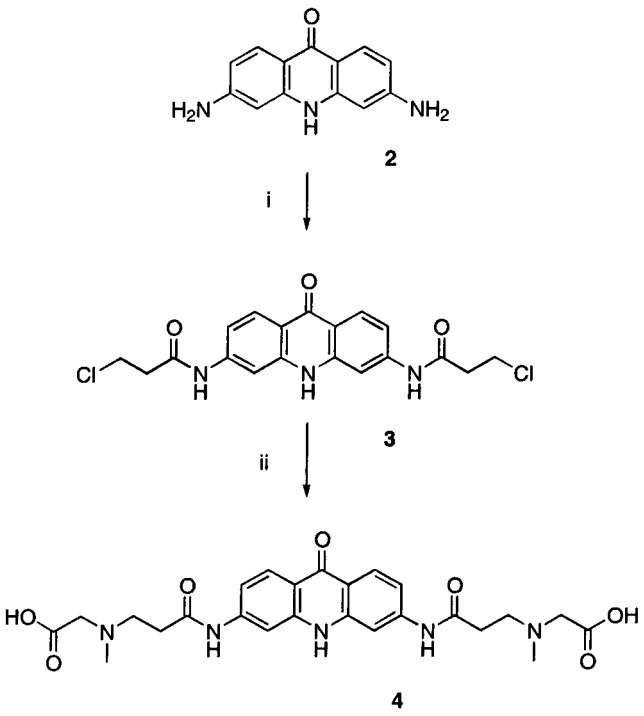
the bifunctional heterocyclic core of interest and two independently linked peptides bound to the same polystyrene bead (Figure 2b).

For our study, we have chosen the 3,6-bisaminoacridone **2** as the bivalent heterocyclic core. It was obtained from the commercially available diphenylmethane, as previously described.[9] Acridone **2** was bisfunctionalized by acylation of the anilino groups with 3-chloropropionyl chloride followed by bis-amination of the dichlorinated derivative with *N*-methylglycine (Scheme 1). Sarcosine was chosen to

Scheme 1[a]



[a] Reagents and conditions: (i) 3-chloropropionyl chloride, reflux, 95%; (ii) sarcosine, NaI, EtOH/H_2O, 90 °C, 3 h, 90%.

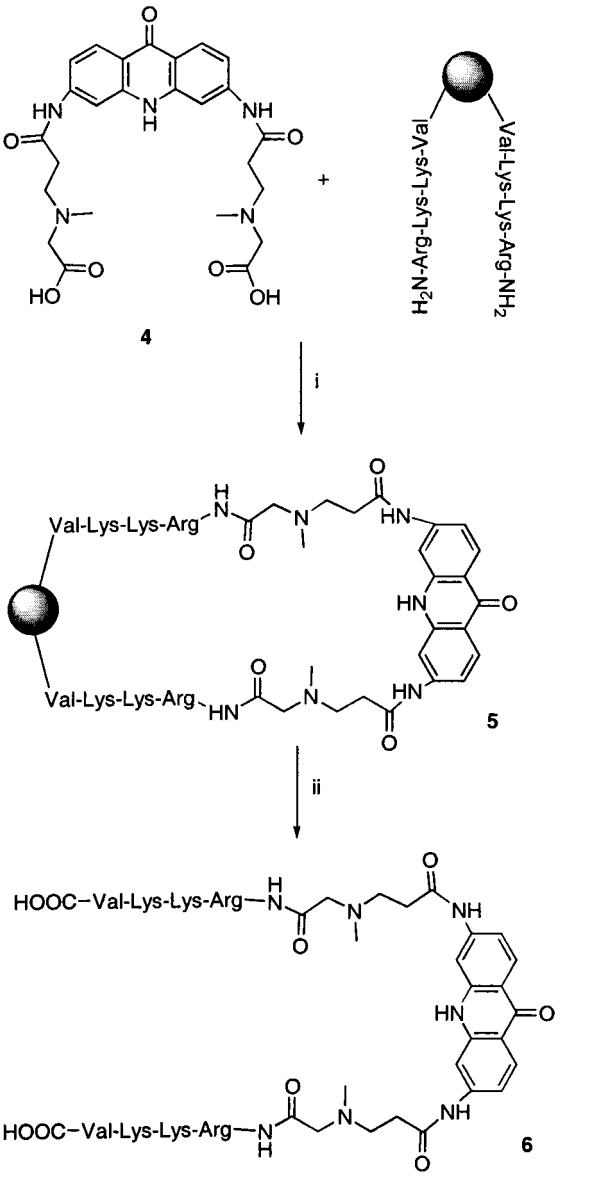
introduce the desired carboxylic acids at the −3 and −6 positions of the heterocyclic core. It also offers the advantage of generating two cationic ternary amines onto the acridone side chains that mimic the cationic pyrrolidine rings of compound **1**.

The synthesis of symmetrical 3,6-bispeptide−acridone conjugates was explored using two chemistries (Scheme 1). Tetrapeptide H_2N-Arg-Lys-Lys-Val-COOH was chosen as a model peptide and was synthesized on PS-Wang resin (initial loading: 0.9 mmol/g, 1% DVB) using standard Fmoc strategy. Following deprotection of the terminal Fmoc group with piperidine, while retaining all side-chain protecting groups, coupling to either the 3,6-bischloroacridone **3** or the 3,6-biscarboxylic acid−acridone **4** was attempted.

Attempts to couple the peptides to compound **3** by direct alkylation under mild conditions failed. Indeed, it was previously reported that addition of secondary amines onto the chloroethylamide groups of **3** in solution required strong conditions (refluxing ethanol, NaI, 12 equiv of amine)[9] that are less convenient for solid-phase chemistry.

However, the carboxylic acids of **4** enabled the coupling of an acridone scaffold to two peptides by amide coupling using PyBOP/HOBt. This bis-coupling was pursued under optimized temperature and reagent concentrations detailed in Scheme 2. All unreacted acridone **4** was easily removed

Scheme 2[a]



[a] Reagents and conditions: (i) 1 equiv of peptide, 2 equiv of **4**, PyBOP−HOBt (20 equiv each), DIPEA (20 equiv), DMSO, 50 °C, 48 h, 85%; (ii) TFA/TIS/H_2O (95:2.5:2.5), 1.5 h, 95%.

by filtration. Unreacted peptide, monopeptide acridone, and the symmetrical bispeptide acridone **5** are all possible resin-bound products.

Simultaneous deprotection of peptide side chains and cleavage from the polystyrene support using TFA/TIS/H_2O

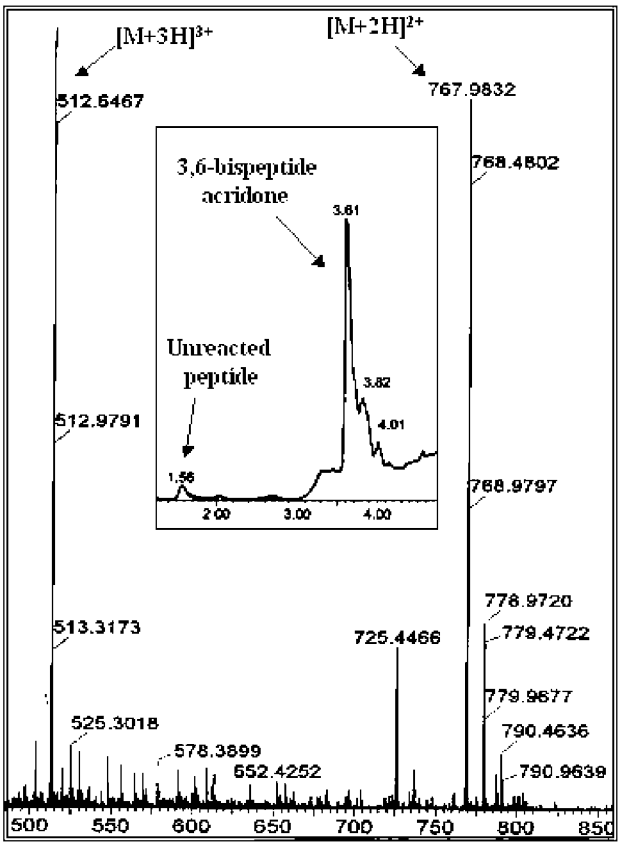


Figure 3. Electrospray mass spectrum of the purified 3,6-bispeptide acridone conjugate **6**. Inset: HPLC trace of the crude post-cleavage product.

(95:2.5:2.5) led to the desired 3,6-bispeptide acridone **6**, which was identified by LC−MS and then purified by HPLC. Under experimental conditions detailed in Scheme 2, the bispeptide acridone **6** was obtained in a purified yield of 80% with respect to the initial loading of tetrapeptide H_2N-Arg-Lys-Lys-Val-COOH. Ten percent unreacted peptide was recovered during the HPLC purification (inset Figure 3), but no monopeptide acridone (product of a single amidic coupling of compound **4** on resin) was detectable.

High-resolution mass spectrometry (HRMS) was used to analyze the HPLC-purified compound **6**. The electrospray mass spectrum (Figure 3) shows two main peaks at m/z = 767.9832 and 512.3170 corresponding to $[M + 2H]^{2+}$ and $[M + 3H]^{3+}$ species, respectively.

This is a clear demonstration and exploitation of on-bead site−site reactivity. Once the acridone derivative **4** has reacted through one of its carboxylate groups with the terminal amino group of a resin-linked, the remaining free carboxylic acid quickly reacts with a second proximal peptide of the same bead.

To test the generality of this methodology, symmetrical bispeptide acridone derivatives of the tetrapeptides H_2N-Lys-Arg-Ser-Arg-COOH, H_2N-Phe-Arg-His-Arg-COOH and the hexapeptide H_2N-Lys-Thr-Arg-Thr-Arg-Ser-COOH were also prepared using the same methodology. For each case, no monopeptide acridone derivative was detected, and the desired bispeptide acridones were obtained in an average yield of 80−85%.

The methodology we report offers the advantage of employing standard protecting group chemistry. Moreover, this multistep synthesis requires only one relatively straightforward HPLC purification step to obtain pure bispeptide−acridone derivatives. This chemistry will be used to generate a library of bispeptide-acridone conjugates for evaluation as G-quadruplex ligands. The general approach may also be useful for the solid-phase synthesis of symmetrical derivatives of other heterocyclic scaffolds.

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Note Added after ASAP Posting. The version of this paper posted on June 26, 2002, had missing bonds in the Abstract, Figure 1, and Scheme 1. The corrected version was posted on June 28, 2002.

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