ORGANIC LETTERS

2000
Vol. 2, No. 10
1465−1468

Solid-Phase Synthesis of Acridine–Peptide Conjugates and Their Analysis by Tandem Mass Spectrometry

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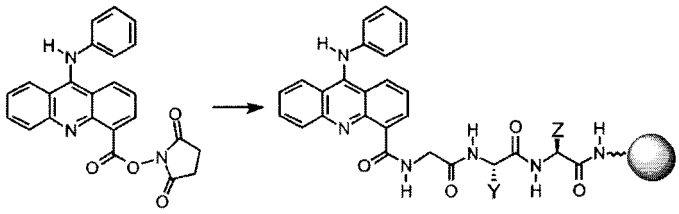
Received March 14, 2000

ABSTRACT



A novel and high-yielding synthesis of 9-anilinoacridine-4-carboxylic acid is reported. This acid has been used in the solid-phase synthesis of a small combinatorial library of acridine–peptide conjugates. Tandem mass spectrometry (ES-MS/MS) can be used for structure determination of these compounds at a sensitivity of ~10 pmol. This work makes possible the generation of acridine–peptide libraries for the discovery of structure-specific nucleic acid ligands via affinity chromatography selection with mass spectrometric detection.

Low molecular weight compounds that bind selectively to nucleic acids and inhibit the formation of protein·nucleic acid complexes have considerable potential as new cancer chemotherapeutics, antiviral or antibacterial agents, and new tools in nucleic acid research.[1−4] Molecules with high affinity and specificity for nucleic acid targets have been discovered via the synthesis and screening of combinatorial libraries biased for DNA or RNA binding.[5−7] For this purpose, we are preparing libraries of nucleic acid binding molecules by elaborating a known intercalating group with a variable peptide appendage. These molecules are designed such that the intercalation domain will direct the peptide into a groove of double helical secondary structure where sequence-specific contacts are possible.

For our initial libraries, we have chosen to prepare acridines with the substitution pattern of SN 16713, a derivative of the antitumor drug amsacrine (Figure 1).[8] SN

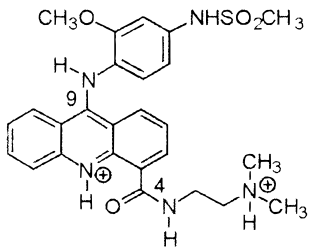


Figure 1. SN 16713, a 9-anilinoacridine-4-carboxamide that binds DNA at G·C-rich sequences and HIV's TAR RNA.

16713 has been shown to bind duplex DNA at G·C-rich sequences and HIV's TAR RNA via intercalation.[9,10] Aniline

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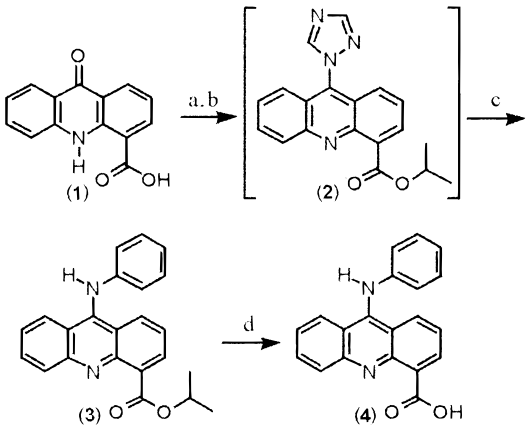
10.1021/ol005809v CCC: $19.00
Published on Web 04/21/2000

substitution at the 9-position of the acridine elevates the ring nitrogen pK_a, such that a significant fraction of the acridine is cationic at physiological pH.[11,12] Carboxamide substitution at the 4-position of acridine has been shown to control DNA binding specificity by allowing for base-specific major groove contacts by the substituent.[13,14] Several 9-aminoacridine-4-carboxamides have been previously prepared.[11,15,16] Typically, the 4-carboxamide bond is formed first by reaction of an amine with 9-chloroacridine-4-carbonyl chloride followed by amine substitution at the 9-position under acidic conditions. This approach is not directly applicable to the solid-phase synthesis of derivatives using standard peptide synthesis protocols. In this Letter, we describe the first reported preparation of 9-anilinoacridine-4-carboxylic acid, its application to the synthesis of 9-anilinoacridine−peptide libraries, and the analysis of these compounds by tandem mass spectrometry (ES-MS/MS).

The known 9(10*H*)-acridone-4-carboxylic acid (**1**), available in two steps from 2-chlorobenzoic and anthranilic acids, was protected as the isopropyl ester via reaction of carbonyldiimidazole (CDI) and 2-propanol in THF (Scheme 1).[17]

Scheme 1[a]

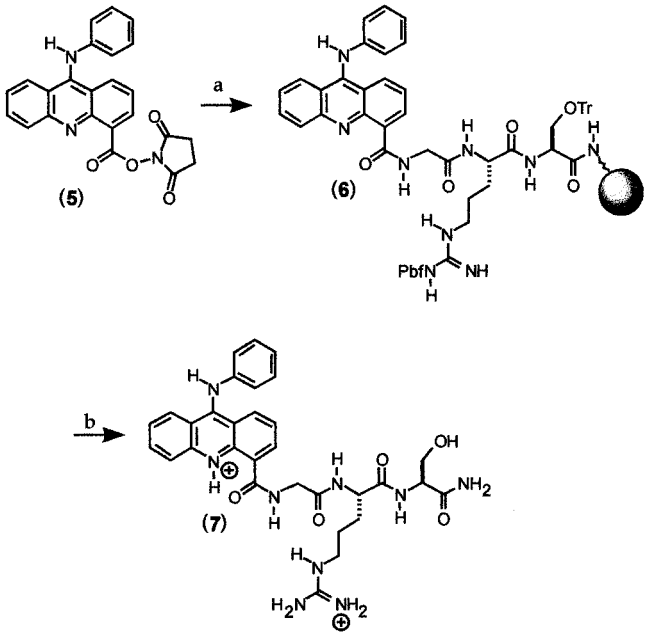


[a] Reagents and conditions: (a) CDI, THF, *iso*-PrOH, rt, 6 h, 95%; (b) 1,2,4-triazole, $POCl_3$, TEA, CH_3CN, reflux, 96 h; (c) aniline, TEA, CH_3CN, reflux, 24 h, 94% (two steps); (d) LiOH, THF, H_2O, rt, 12 h, 99%.

The ester was then subjected to reaction conditions known to convert oxygen-substituted heterocycles to 1,2,4-triazoles.[18] This reaction has been used successfully in the synthesis of nucleoside analogues from uridine and thymidine derivatives.[19] The triazole intermediates formed react selectively with a number of different nitrogen and oxygen nucleophiles under mildly basic conditions to give substitution products in good yield. We investigated the utility of this reaction for substitution of the acridine ring because we believed these conditions would be compatible with the acridone-4-carboxylic acid protected as a hindered ester. Upon treatment of the acridone isopropyl ester with an acetonitrile solution of the triazolating reagent formed from 1,2,4-triazole, $POCl_3$, and TEA, the starting material was slowly consumed and a new product, presumably the 9-triazoleacridine derivative (**2**), was formed. This compound was not isolated but was allowed to react with aniline in acetonitrile at reflux in the presence of TEA to give ester **3** in high yield. Ester deprotection with LiOH in THF/H_2O gave acid **4** in 88% overall yield from **1**.

To determine if acid **4** could be used directly in solid-phase synthesis of acridine−peptide conjugates, it was applied to the preparation of a 9-anilinoacridine-4-carboxamide with a short peptide fused to the acridine through a 4-carboxamide linkage to the N-terminus. The tripeptide H_2N-Gly-Arg-Ser-COOH was synthesized using standard Fmoc peptide synthesis procedures on Rink amide resin. Acid **4** was activated as the NHS ester **5**, and this compound was allowed to react with the free amino terminus of the side chain-protected, solid support-bound peptide to give **6** (Scheme 2). Removal of unreacted **5** by filtration was

Scheme 2[a]



[a] Reagents and conditions: (a) resin-bound peptide + **5** (3 equiv), THF, rt, 12 h; (b) TFA:TIS:H_2O (95:2.5:2.5), rt, 18 h.

followed by deprotection of the side chains and cleavage from the support with TFA:TIS:H_2O, giving acridine−

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peptide conjugate **7**. Tandem mass spectrometry (ES-MS/MS) was used to analyze this compound (Figure 2). The

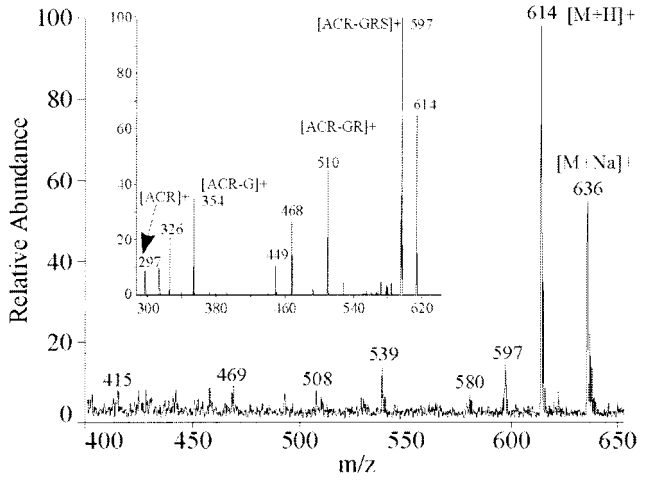

Figure 2. Electrospray mass spectrum of the 9-anilinoacridine−peptide conjugate ACR−GRS (**7**). Inset: Daughter ion analysis of $m/z = 614$ peak.

electrospray mass spectrum showed a peak at $m/z = 614$, corresponding to $[M + H]^+$. Fragmentation of this ion generated daughter ions at 597, 510, 354, and 297, corresponding to cleavage at the four amide bonds in the molecule (Figure 2, inset).

Importantly, these results indicate that the presence of the 9-anilinoacridine does not interfere with mass spectrometry sequencing of these peptide conjugates. Furthermore, the sensitivity of the ES-MS/MS technique (~10 pmol per peptide) allows for the determination of their structures with very little material. For comparison, a typical peptide synthesis with 0.2 g of Rink amide resin corresponds to 80−160 μmol of peptide.

Using a split and pool strategy with Fmoc-protected amino acids,[20] Rink amide resin, and NHS ester **5**, a small acridine−peptide library was prepared. This library had one fixed and two randomized positions. At each randomized position, four different amino acids (Ala, Gly, Ser, and Lys) were incorporated to give 16 sequences. After deprotection of the N-terminal glycine, **5** was allowed to react with the library. Removal of unreacted ester by filtration, side chain deprotection, and cleavage from the support yielded the acridine−peptide library **8** (Figure 3). ES-MS/MS was used to analyze components of approximately 120 pmol of this library (Figure 4). Nine of the 10 different masses expected for the 16 components of this library were detected as the $[M + H]^+$ ions in the electrospray mass spectrum. The GKK component was detected as the $[M + Na]^+$ adduct. In

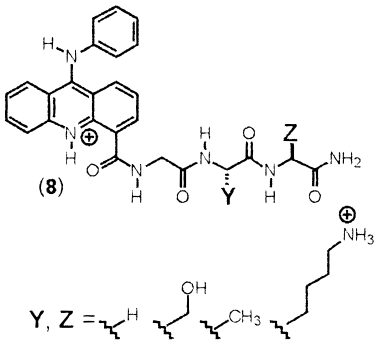

Figure 3. Structure of a small combinatorial library of 9-anilinoacridine−peptides. The tripeptide appendage has one fixed residue (G) and two randomized positions, Y and Z, each with four different amino acids (A, G, K, and S).

addition to the singly charged ions shown, the lysine-containing conjugates were also detected as the $[M + 2H]^{2+}$ ions (data not shown). The ion at $m/z = 545$ was selected for daughter ion analysis and the result confirmed its identity as the 9-anilinoacridine−GSS conjugate (Figure 4, inset).

In summary, we have developed a high-yielding synthesis to 9-anilinoacridine-4-carboxylic (**4**), and this acid has been applied to the solid-phase synthesis of 9-anilinoacridine-4-carboxamides. Peptides linked to acridine via a 4-carboxamide linkage can be readily prepared using standard Fmoc peptide synthesis techniques and the NHS ester of **4**. We have shown that structures of these acridine−peptide conjugates can be determined with less than 10 pmol of compound by tandem mass spectrometry. We are currently screening 9-anilinoacridine−peptide libraries prepared with these methods by affinity chromatography selection with

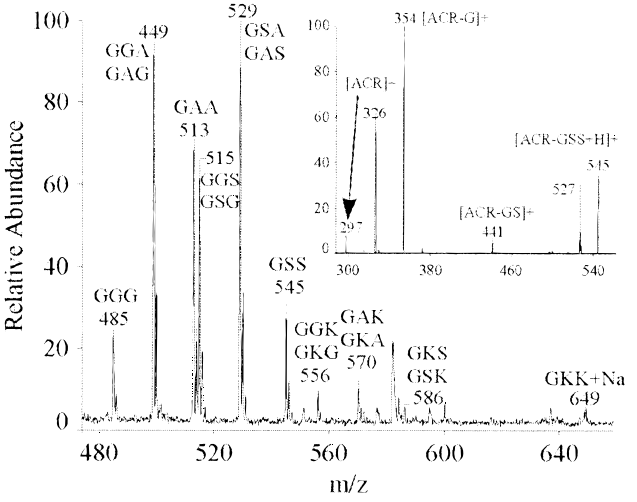

Figure 4. ES-MS/MS fragmentation pattern of the 9-anilinoacridine−peptide library, indicating that masses corresponding to the 16 components are detected. The peak at $m/z = 582$ represents an unknown impurity. Inset: daughter ion analysis of $m/z = 545$ peak.

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mass spectrometric detection to identify members that bind with high affinity and selectivity to predefined nucleic acid structures.[21]

Acknowledgment. P.A.B acknowledges support from the University of Utah Research Foundation. C.B.C was supported by a National Institutes of Health training grant (GM-08573). The authors also thank Dr. Vajira Nanayakkara of the University of Utah mass spectrometry facility for his assistance and Professor James McCloskey for helpful discussions.

Supporting Information Available: Experimental procedures and NMR and mass spectral data for compounds listed in Scheme 1. Additionally, the general procedure for the synthesis and ES-MS/MS analysis of a representative 9-anilinoacridine−peptide conjugate is included. This information is available free of charge via the Internet at http://pubs.acs.org.

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