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Solid-Phase Synthesis of Acridine-Based Threading Intercalator Peptides

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Abstract—The preparation of a novel acridine-based amino acid is reported. This *N*-Alloc-protected monomer can be coupled and deprotected under solid-phase peptide synthesis procedures to create acridine–peptide conjugates as potential threading intercalators. A peptide containing this novel amino acid undergoes spectral changes in the presence of duplex DNA and RNA consistent with intercalative binding.

Solid-phase synthesis of combinatorial libraries, affinity chromatography and mass spectrometry are experimental techniques that have converged to create a productive approach to the discovery of small molecules that bind to biological receptors.[1,2] Our laboratory is using this approach in the search for compounds that bind with high affinity and specificity to nucleic acids.[3] Such compounds are of interest as potential therapeutic agents and new tools in nucleic acid biochemistry.[4–7]

The primary modes of noncovalent binding of small molecules to DNA and RNA are intercalation, groove binding, coulombic attraction or a combination of these.[7,8] Threading intercalation is a binding mode wherein the intercalating group directs substituents into both grooves of a duplex simultaneously.[9] This typically requires a bulky side chain to pass between base pairs, or thread, during the binding event. A number of compounds have been shown to bind DNA by the threading mechanism, including 9-anilinoacridine-4-carboxamides.[10] In the intercalation complex, acridine substituents at the 4- and 9-positions are located in the major and minor grooves with the acridine chromophore sandwiched between base pairs (Fig. 1). This binding mode allows for base-specific groove binding by the substituents to take place. Indeed, the 4-substituent of a 9-aminoacridine-4-carboxamide derivative was recently shown by X-ray crystallography to make specific contacts in the major groove of a DNA double helix.[11,12]

The ability of threading intercalators to direct functionality into both the major and minor grooves of nucleic acids makes them good candidates for selective ligands. These grooves provide unique molecular recognition surfaces that can be exploited to distinguish different nucleic acid structures.

Many derivatives of acridine substituted at both the 4- and 9-positions have been synthesized.[13–15] We recently reported the preparation of combinatorial libraries of acridine–peptide conjugates by modifying a 9-anilinoacridine with a variable peptide appendage at the 4-position.[3] We are currently screening these libraries, whose major groove appendage is randomized, for structure–specific nucleic acid ligands via affinity chromatography selection with mass spectrometric detection. To advance these studies further, we devised a synthetic route to attach a variable peptide appendage at the 9-position of an acridine intercalator. In this letter, we report the synthesis of 9-(4′-methylamino-allyloxycarbamate)-anilinoacridine-4-carboxylic acid **1**, a non-natural acridine-based amino acid, and its incorporation into a fixed peptide sequence. This peptide demonstrated spectral changes upon titration with either duplex DNA or RNA consistent with intercalative binding. Thus, using this new amino acid and solid-phase peptide synthesis (SPPS), acridine derivatives can be rapidly prepared that place peptide appendages at the 4- and 9-positions, which are directed into the grooves of nucleic acid duplexes in intercalation complexes.

Synthesis

The synthesis of this novel amino acid (Scheme 1) first required selective protection of the primary amino group of 4-aminobenzylamine. We chose the Alloc

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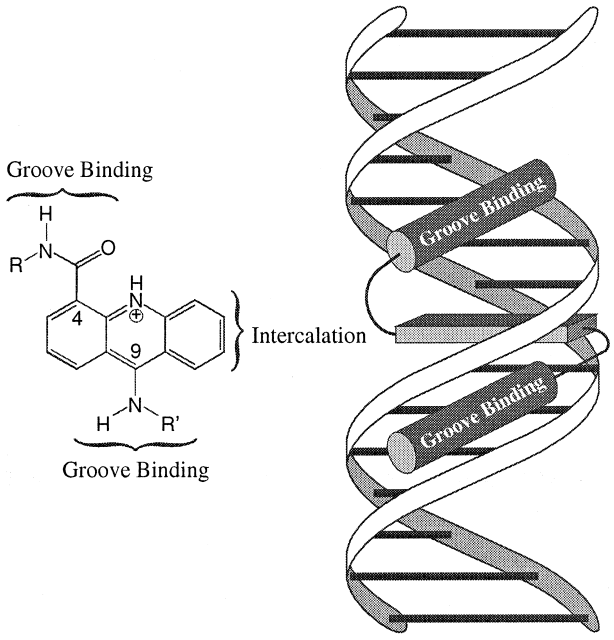
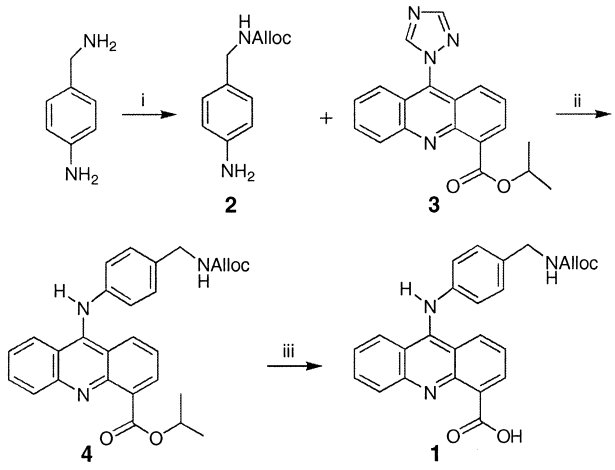


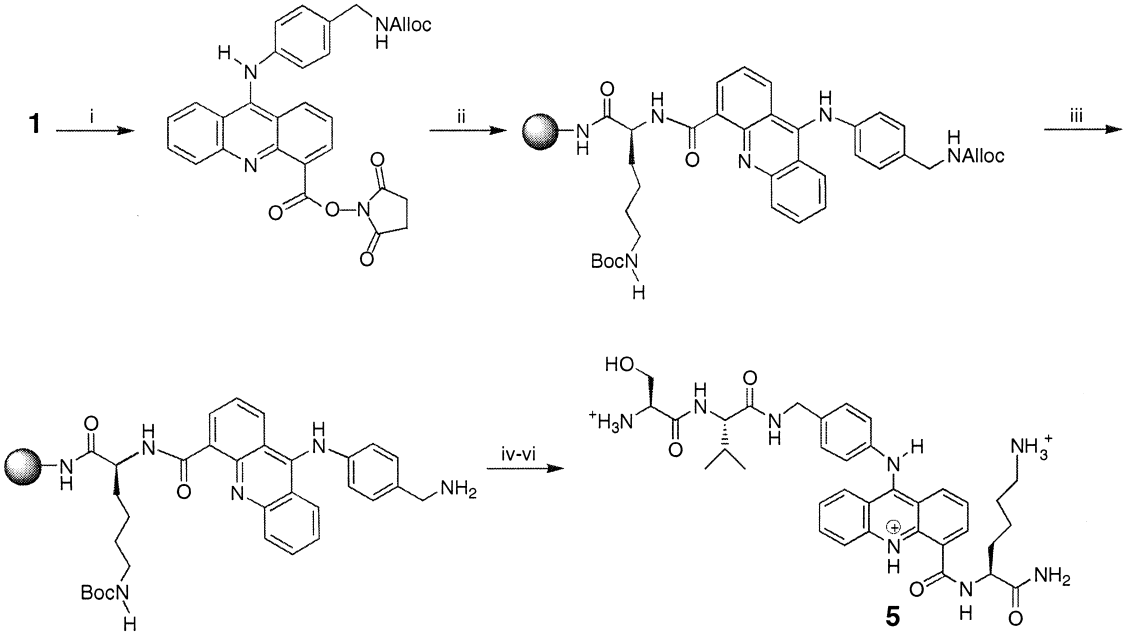
Figure 1. Generalized structure of 4,9-disubstituted acridines and schematic of their binding to duplex DNA by threading intercalation.



Scheme 1. Preparation of Alloc-protected amino acid (**1**).[19] (i) allyl 1-benzotriazolyl carbamate/TEA/THF/rt/6 h/92%; (ii) TEA/CH_3CN/reflux/24 h/75%; (iii) LiOH/H_2O/THF/rt/12 h/72%.

group for two reasons. First, we needed a base stable protecting group for our conditions of 9-substitution of the acridine ring. Secondly, we desired a protecting group that could be removed under conditions that would leave acid-labile side chain protecting groups intact, allowing this amino acid to be incorporated at any point during SPPS using *N*-α-Fmoc amino acids. The protection was accomplished using allyl-1-benzotriazolyl carbonate to yield the Alloc-protected amine **2** in 92% yield.[16] Reaction of **2** with acridine ester **3**, synthesized previously in our laboratory,[3] gave compound **4** in high yield.[17] Subsequent saponification of the ester afforded *N*-Alloc-protected amino acid **1**.[18]

To demonstrate that **1** could be used in SPPS, the fixed peptide sequence *N*-Ser-Val-Acr-Lys-C was prepared, where Acr refers to the 4,9-disubstituted acridine (Scheme 2). Amino acid **1** was activated as the *N*-hydroxysuccinimide (NHS) ester and coupled to the solid-supported primary amino group of *N*-ε-Boc-lysine. To remove the Alloc group and reveal the benzyl amine for subsequent elaboration by peptide synthesis, we chose a set of conditions similar to those previously reported to be compatible with resin-bound peptides ($Pd(PPh_3)_4$ in $CHCl_3$:AcOH:NEM, rt, 2 h).[20] Monitoring the efficiency



Scheme 2. Incorporation of acridine-based amino acid (**1**) into the fixed peptide sequence N-Ser-Val-Acr-Lys-C (**5**). (i) NHS/DCC/THF/rt/12 h; (ii) Rink Amide AM resin-supported *N*-ε-Boc-lysine/TEA/rt/12 h; (iii) $Pd(PPh_3)_4/CHCl_3$:AcOH:NEM (37:2:1)/rt/2 h; (iv) SPPS incorporation of Fmoc-Val-OH; (v) SPPS incorporation of Fmoc-Ser(Trt)-OH; (vi) TFA:TIS:H_2O (95:2.5:2.5)/rt/18 h.

of Alloc removal in our case was not possible by the usual colorimetric assays, since the beads took on an orange color themselves upon coupling of the acridine amino acid to the resin. Instead, essentially quantitative Alloc removal under the above conditions was confirmed by spectroscopic evaluation of Fmoc release after the subsequent amino acid coupling. Upon completion of the solid-phase synthesis, the tetrapeptide was released and the side chains were deprotected by treatment of the resin with TFA:TIS:H_2O (95:2.5:2.5) to give **5**. The structure of **5** was confirmed by electrospray mass spectrometry (Fig. 2). Tandem mass spectrometric analysis (ES-MS/MS) of m/z peak 657.3 gave daughter ion peaks consistent with the expected fragmentation pattern of this peptide.[21]

Nucleic Acid Binding

The binding of intercalators to nucleic acids has been studied by a number of methods.[22] These experiments usually evaluate the structural changes in the helix or the spectroscopic changes of the chromophore. Acridines have been shown to undergo bathochromic and hypochromic shifts in their visible absorption spectra upon binding DNA.[23] To determine if such changes could be observed with **5**, calf thymus DNA was titrated into a buffered aqueous solution of **5**. A bathochromic shift (12 nm) and decrease in extinction coefficient was observed for the visible absorption maximum (Fig. 3).

The magnitudes of these changes are similar to those observed when other 9-anilinoacridine-4-carboxamides bind calf thymus DNA.[24] Upon titration with poly-I•polyC, a similar shift and change in extinction coefficient was observed, indicating that **5** can bind both B-form and A-form helices (data not shown).[25] These experiments illustrate that the chemical modifications made to the acridine ring system did not prevent its interaction with DNA or RNA. Although these results are not proof of intercalation by **5**, they are consistent with intercalative binding.[26] Furthermore, they indicate that this acridine–peptide conjugate binds in a manner similar to acridine

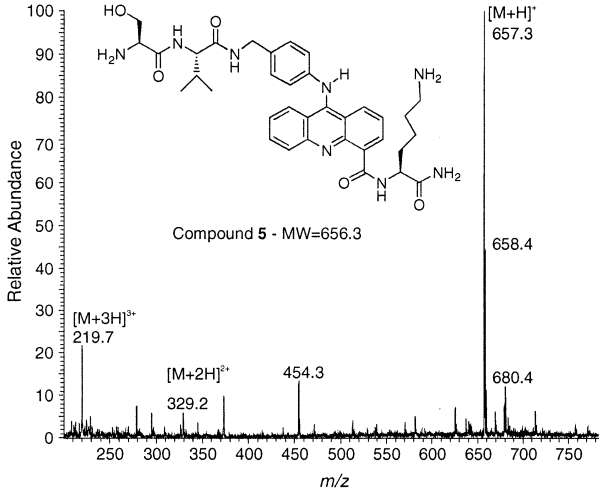


Figure 2. Electrospray mass spectrum of intercalator peptide *N*-Ser-Val-Acr-Lys-C after reverse-phase HPLC purification.

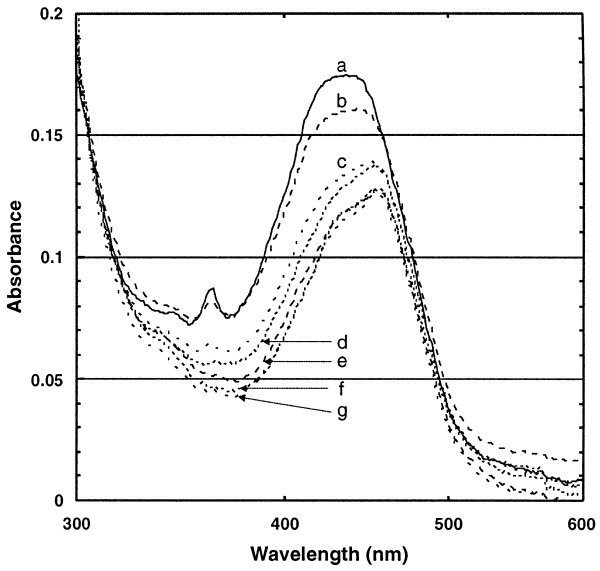


Figure 3. UV–visible spectrum of **5** (30 μM in 0.01 M PIPES buffer pH 7, 0.01 M NaCl, 1 mM EDTA) upon titration with calf thymus DNA. (a) no DNA (unbound, $\lambda_{max} = 442$ nm); (b) 10 μM base pairs (bp); (c) 20 μM bp; (d) 30 μM bp; (e) 40 μM bp; (f) 50 μM bp; (g) 60 μM bp (bound, $\lambda_{max} = 454$ nm).

derivatives that have been shown to bind via intercalation by other experimental techniques.[12,27,28]

Conclusion

We have reported the preparation of an acridine-based amino acid derivative and its incorporation into a peptide using solid-phase peptide synthesis. The structure of this compound is reminiscent of known threading intercalators, such as other 9-anilinoacridine-4-carboxamides.[10] A peptide containing this nonnatural amino acid undergoes spectral changes in the presence of DNA and RNA consistent with intercalative binding. The synthetic approach reported here may be a useful drug-design strategy, since other 9-anilinoacridines have been shown to be potent inhibitors of the cancer chemotherapy target topoisomerase II.[29,30] Furthermore, threading intercalators have the potential for regiospecific delivery of variable functional groups to the two different grooves of DNA or RNA duplexes. Both of these characteristics make these intercalator peptides good candidates for future study.

Acknowledgements

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References and Notes

1. Kelly, M. A.; Liang, H.; Sytwu, I.-I.; Vlattas, I.; Lyons, N. L.; Bowen, B. R.; Wennogle, L. P. *Biochemistry* **1996**, *35*, 11747.

2. Huang, P. Y.; Carbonell, R. G. *Biotech. Bioeng*. **1999**, *63*, 633.
3. Carlson, C. B.; Beal, P. A. *Org. Lett*. **2000**, *2*, 1465.
4. Li, K.; Fernandez-Saiz, M.; Rigl, C. T.; Kumar, A.; Ragunathan, K. G.; McConnaughie, A. W.; Boykin, D. W.; Schneider, H.-J.; Wilson, W. D. *Bioorg. Med. Chem*. **1997**, *5*, 1157.
5. Michael, K.; Tor, Y. *Chem. Eur. J*. **1998**, *4*, 2091.
6. Pearson, N. D.; Prescott, C. D. *Chem. Biol*. **1997**, *4*, 409.
7. Nielsen, P. E. *Bioconj. Chem*. **1991**, *2*, 1.
8. Bogdan, F. M.; Chow, C. S. *Chem. Rev*. **1997**, *97*, 1489.
9. Liaw, Y.-C.; Gao, Y.-G.; Robinson, H.; van der Marel, G. A.; van Boom, J. H.; Wang, A. H.-J. *Biochemistry* **1989**, *28*, 9913.
10. Wakelin, L. P. G.; Chetcuti, P.; Denny, W. A. *J. Med. Chem*. **1990**, *33*, 2039.
11. Todd, A. K.; Adams, A.; Thorpe, J. H.; Denny, W. A.; Wakelin, L. P. G. *J. Med. Chem*. **1999**, *42*, 536.
12. Adams, A.; Guss, J. M.; Collyer, C. A.; Denny, W. A.; Wakelin, L. P. G. *Biochemistry* **1999**, *38*, 9221.
13. Denny, W. A.; Cain, B. F.; Atwell, G. J.; Hansch, C.; Panthananickal, A.; Leo, A. *J. Med. Chem*. **1982**, *25*, 276.
14. Bourdouxhe-Housiaux, C.; Colson, P.; Houssier, C.; Waring, M. J.; Bailly, C. *Biochemistry* **1996**, *35*, 4251.
15. Bourdouxhe-Housiaux, C.; Colson, P.; Houssier, C.; Bailly, C. *Anti-Cancer Drug Des*. **1996**, *11*, 509.
16. Preparation of allyl 4-aminobenzylamino carbamate (**2**). To a mixture of allyl 1-benzotriazolyl carbamate (0.580 g, 2.65 mmol, 1.2 equiv) in THF (2.5 mL), was added dropwise 4-aminobenzylamine (0.250 mL, 2.21 mmol) and TEA (0.260 mL, 1.2 equiv). The resulting yellow solution was stirred at rt for 6 h. When the reaction was complete by thin layer chromatography (TLC) (acetone:TEA 98:2), the solvent was evaporated under reduced pressure. The residual liquid was purified by flash chromatography on a column of silica gel, eluting with EtOAc:hexanes (3:2) to give 0.390 g (92%) of protected amine **1** as a viscous liquid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 7.01 (d, 2H, J = 8.4 Hz), 6.56 (d, 2H, J = 8.4 Hz), 5.89 (m, 1H), 5.51 (bs, 1H amide), 5.26 (dd, 1H, J = 17.1, 1.5 Hz), 5.18 (dd, 1H, J = 10.2, 1.5 Hz), 4.54 (d, 2H, J = 6 Hz), 4.18 (d, 2H, J = 5.7 Hz), 3.75 (s, 2H aniline); FABHRMS calcd mass for $C_{11}H_{15}N_2O_2$: 207.124, found 207.123.
17. Preparation of *iso*-propyl-9-(4′-methylaminoallyl-oxycarbamate)anilinoacridine-4-carboxylate (**4**). To a solution of ester **3** (0.75 mmol) in anhydrous acetonitrile (8 mL), was added amine **1** (3 mmol, 4 equiv) and TEA (1 mL, 7.5 mmol, 10 equiv). The resulting mixture was heated under reflux for 24 h. When the reaction was complete by TLC (EtOAc:hexanes:MeOH 90:9:1), the solution was allowed to cool to rt, and solvent was evaporated under reduced pressure. The residue was purified by flash chromatography on a column of alumina oxide, eluting with hexanes:EtOAc (4:1) to give 0.262 g (75%) of ester **4** as an orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 11.32 (bs, 1H), 8.24 (bs, 1H), 7.35 (d, 1H), 7.25 (d, 2H), 7.18 (d, 2H), 6.82 (d, 2H), 5.96 (m, 1H), 5.33 (dd, 1H), 5.30 (m, 1H *iso*-propyl methine), 5.23 (dd, 1H), 5.11 (bs, 1H), 4.63 (d, 2H), 4.39 (d, 2H), 1.44 (d, 6H); FABHRMS calcd mass for $C_{28}H_{28}N_3O_4$: 470.206, found 470.208.
18. Preparation of 9-(4′-methylamino-allyloxycarbamate)-anilinoacridine-4-carboxylic acid (**1**). To a solution of ester **3** (0.250 g, 0.533 mmol) in THF (5 mL), was added lithium hydroxide monohydrate (0.046 g, 1.1 mmol, 2 equiv) in water (0.6 M). The resulting solution was stirred at rt for 12 h. The reaction solution was neutralized with 2N HCl, and the solvent was removed under reduced pressure. The brick red residual oil was purified by flash chromatography (EtOAc:hexanes 9:1 w/ late addition of methanol) on a column of silica gel to give 0.163 g (72%) of acid **1** as a reddish-orange solid. ^{1}H NMR (500 MHz, $CDCl_3$) δ 8.40 (bs, 1H), 8.25 (bs, 1H), 7.94 (bs, 1H), 7.77 (t, 1H), 7.69 (bs, 2H), 7.22 (m, 3H), 6.93 (bs, 2H), 5.92 (m, 1H), 5.29 (dd, 1H), 5.18 (dd, 1H), 4.51 (d, 2H), 4.20 (d, 2H); FABHRMS calcd mass for $C_{25}H_{22}N_3O_4$: 428.161, found 428.161.
19. Abbreviations used in the synthetic schemes are: AcOH, acetic acid; DCC, 1,3-dicyclohexylcarbodiimide; NEM, *N*-ethylmorpholine; NHS, *N*-hydroxysuccinimide; TEA, triethylamine; TFA, trifluoroacetic acid; THF, tetrahydrofuran; TIS, triisopropylsilane.
20. Kates, S. A.; Daniels, S. B.; Sole, N. A.; Barany, G. In *Peptides, Chemistry, Structure & Biology, Proc. 13th American Peptide Symposium*; Hodges, R. S., Smith, J. A., Eds.; ESCOM: Leiden, 1994; Vol. 13, pp 113–115.
21. ES-MS/MS data for **5**: *m*/*z* 657.3 (N-Ser-Val-Acr-Lys-C); 570.4 (N-Val-Acr-Lys-C); 512.2 (N-Ser-Val-Acr-C); 425.2 (*N*-Val-Acr-C); 326.1 (*N*-Acr-C).
22. Long, E. C.; Barton, J. K. *Acc. Chem. Res*. **1990**, *23*, 273.
23. Denny, W. A.; Wakelin, L. P. G. *Cancer Res*. **1986**, *46*, 1717.
24. Searcey, M.; Martin, P. N.; Howarth, N. M.; Madden, B.; Wakelin, L. P. G. *Bioorg. Med. Chem. Lett*. **1996**, *6*, 1831.
25. The RNA-binding experiment was conducting in the same fashion as for DNA. Titration of compound **5** (30 μM in 0.01 M PIPES buffer pH 7, 0.01 M NaCl, 1 mM EDTA) with polyI•polyC RNA (10 μM bp increments). A bathochromic shift of 10 nm was observed and saturation was reached at approximately the same concentration as with DNA (60 μM).
26. As are many acridine derivatives, compound **5** is fluorescent, with excitation and emission maxima at 400 and 450 nm, respectively. The emission maximum was blue-shifted and the intensity decreased in the presence of excess calf thymus DNA, again consistent with intercalation.
27. Westhof, E.; Sundaralingam, M. *Proc. Natl. Acad. Sci. U.S.A*. **1980**, *77*, 1852.
28. Sakore, T. D.; Reddy, B. S.; Sobell, H. M. *J. Mol. Biol*. **1979**, *114*, 763.
29. Figgitt, D. P.; Denny, W. A.; Gamage, S. A.; Ralph, R. K. *Anti-Cancer Drug Des*. **1994**, *9*, 199.
30. Froelich-Ammon, S. J.; Osheroff, N. *J. Biol. Chem*. **1995**, *270*, 21429.