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Peptide Quinoline Conjugates: A New Class of RNA-Binding Molecules

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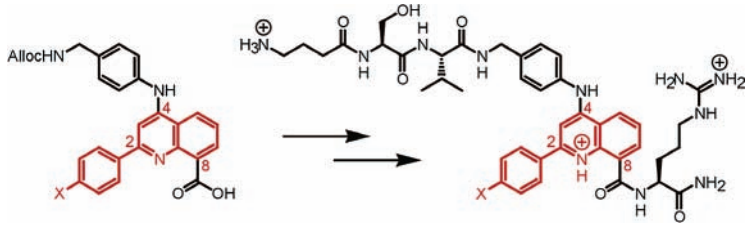
ABSTRACT



A synthesis of 4,8-disubstituted 2-phenylquinoline amino acids is reported with the incorporation of one example into a peptide by solid-phase synthesis. The phenylquinoline-containing peptide binds an RNA target with nanomolar affinity (K_D = 208 nM). The strategy can be used to prepare a variety of 2-substituted quinoline amino acids for alteration of affinity in intercalator peptides. Since quinolones represent an important class of antibacterials, these compounds may be useful in the discovery of new antibacterial agents.

The appreciation of RNA as a target for new antibiotic drugs has increased interest in the development of low molecular weight compounds that bind RNA selectively.[1−5] Early studies indicate the major challenge in developing small molecule ligands for RNA is making them specific for a given RNA target.[6,7] Compounds that bind an RNA structure and make contacts at several sites using a variety of binding modes (e.g., π-stacking, H-bonding, van der Waals contacts, charge−charge interactions, etc.) will likely be the most selective, since other RNAs will not have the requisite arrangement of complementary functional groups. A particularly attractive binding mechanism that allows for multiple points of contact between ligand and RNA is threading intercalation.[8−11] Threading intercalation involves insertion of an intercalator between base pairs of a nucleic acid duplex and localization of distinguishable substituents on the heterocycle in opposite grooves of the helix. We have reported a solid-phase synthesis approach to threading intercalator peptides with an acridine chromophore introduced into the peptide backbone such that the N- and C-termini lie in opposite grooves in an intercalation complex.[12] This design allows the groove-localized substituents to be varied via combinatorial peptide synthesis.

Importantly, for selective binding to be achieved, the relative contribution to the binding affinity of functional groups in the ligand must be balanced to prevent nonspecific binding to imperfectly matched sites. In the case of threading intercalators, if the affinity of the intercalating group is too large relative to other groups in the ligand, additional nucleic acids with accessible π-stacking surfaces may also be bound with similar affinities. Thus, controlling the binding affinity of the intercalating group will become increasingly important as threading intercalators are developed for selective RNA binding. In this Letter, we describe a new threading inter-

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calator design: the peptide quinoline conjugate (PQC). A solid-phase synthesis scheme is reported for the preparation of 2-phenylquinolines with peptide substituents at the 4- and 8-positions. Furthermore, the ability of the intercalator to π-stack can be readily modulated by altering the polarity of the π-aromatic system through substitution of the 2-phenyl group. The RNA binding properties of a prototypical PQC are also evaluated.

To generate quinoline-based threading intercalator peptides, we prepared compounds **1** and **2**, each with an Alloc-protected 4-aminobenzylamine appendage at the quinoline 4-position and a carboxyl group at the 8-position (Figure 1). A phenyl group is present at position 2, since 2-phenyl-

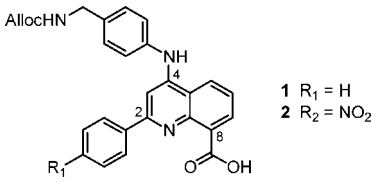
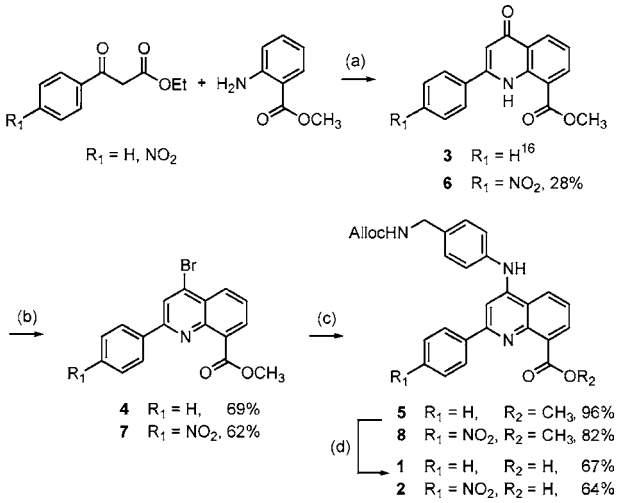


Figure 1. Amino acids synthesized for incorporation into peptide quinoline conjugates via SPPS.

quinolines are known intercalators. Furthermore, the π-stacking ability of the 2-phenyl group present in compound **2** is modulated by the polarizing nitro substituent. In analogy to the acridine-containing amino acid previously described by us, these compounds are likely to be compatible with solid-phase peptide synthesis (SPPS) with Fmoc-protected amino acids.[12,13] 2-Phenyl-4-aminoquinoline derivatives and 2-phenylquinoline-8-carboxamides have been shown to bind nucleic acids by intercalation.[14,15] However, there are no prior reports of 2-phenylquinolines simultaneously modified at positions 4 and 8 in this manner. This substitution pattern creates molecules with substituents on opposite edges of the quinoline heterocycle and, thus, potential threading intercalators.

Our synthesis of **1** begins with 8-methoxycarbonyl-2-phenyl-4(1*H*)-quinolone **3**, which is prepared as described by Denny via condensation of methyl anthranilate and ethylbenzoyl acetate followed by thermal cyclization of the condensation product (Scheme 1).[16] Bromination of **3** by treatment with triphenylphosphine and *N*-bromosuccinimide in acetonitrile gave **4** in good yield.[17] Given our recent experience with simple substitution reactions involving acetyl-protected bromopurine ribonucleosides, we anticipated that bromoquinoline **4** would react selectively at the 4-position under mild conditions with nitrogen nucleophiles, even

Scheme 1 [a]



[a] (i) CH_3SO_3H/benzene reflux, (ii) Ph_2O/255 °C; (b) PPh_3/NBS/CH_3CN reflux; (c) allyl 4-aminobenzyl-amino carbamate[12]/CH_3CN reflux; (d) $LiOH{\cdot}H_2O$/THF−H_2O rt.

weakly nucleophilic aniline derivatives.[18] Indeed, compound **4** is readily converted to 4-anilino derivative **5** in acetonitrile at reflux in excellent yield. Saponification of the methyl ester gave *N*-Alloc-protected amino acid **1** ready for activation and coupling in SPPS. This same strategy can be extended to prepare quinoline amino acids with a substituted phenyl group of varying π-system polarity, as indicated by the synthesis of nitro compound **2** (Scheme 1). The synthesis of **2** was initiated by condensation of ethyl(4-nitrobenzoyl)-acetate and methylanthranilate followed by cyclization to give quinolone **6**. Bromination to **7**, substitution with Alloc-protected amine to give **8**, and saponification produced 2-(4-nitrophenyl)quinoline amino acid **2**.

To demonstrate that these 2-phenylquinoline amino acids can be used in SPPS, the peptide sequence *N*-Abu-Ser-Val-PheQ-Arg-*C* was prepared, where Abu is aminobutyric acid and PheQ refers to the 4,8-disubstituted 2-phenylquinoline amino acid. Amino acid **1** was activated as the *N*-hydroxy-succinimide (NHS) ester and coupled to the solid-supported primary amino group of Pbf-protected arginine. The Alloc group was removed using $Pd(PPh_3)_4$/$PhSiH_3$ in CH_2Cl_2 at room temperature for 30 min to reveal the benzylamine for subsequent elaboration by peptide synthesis.[19] The additional amino acid residues were introduced via standard protocols. Upon completion of the solid-phase synthesis, the peptide was released and the side chains were deprotected by treatment of the resin with TFA/TIS/PhOH/H_2O (88:5:5:2) to give peptide **9** (Figure 2). The structure of **9** was confirmed by fast atom bombardment and electrospray ionization mass spectrometry.

The threading peptide *N*-Abu-Ser-Val-Acr-Arg-*C* (**10**), where Acr is a 4,9-disubstituted acridine amino acid, binds

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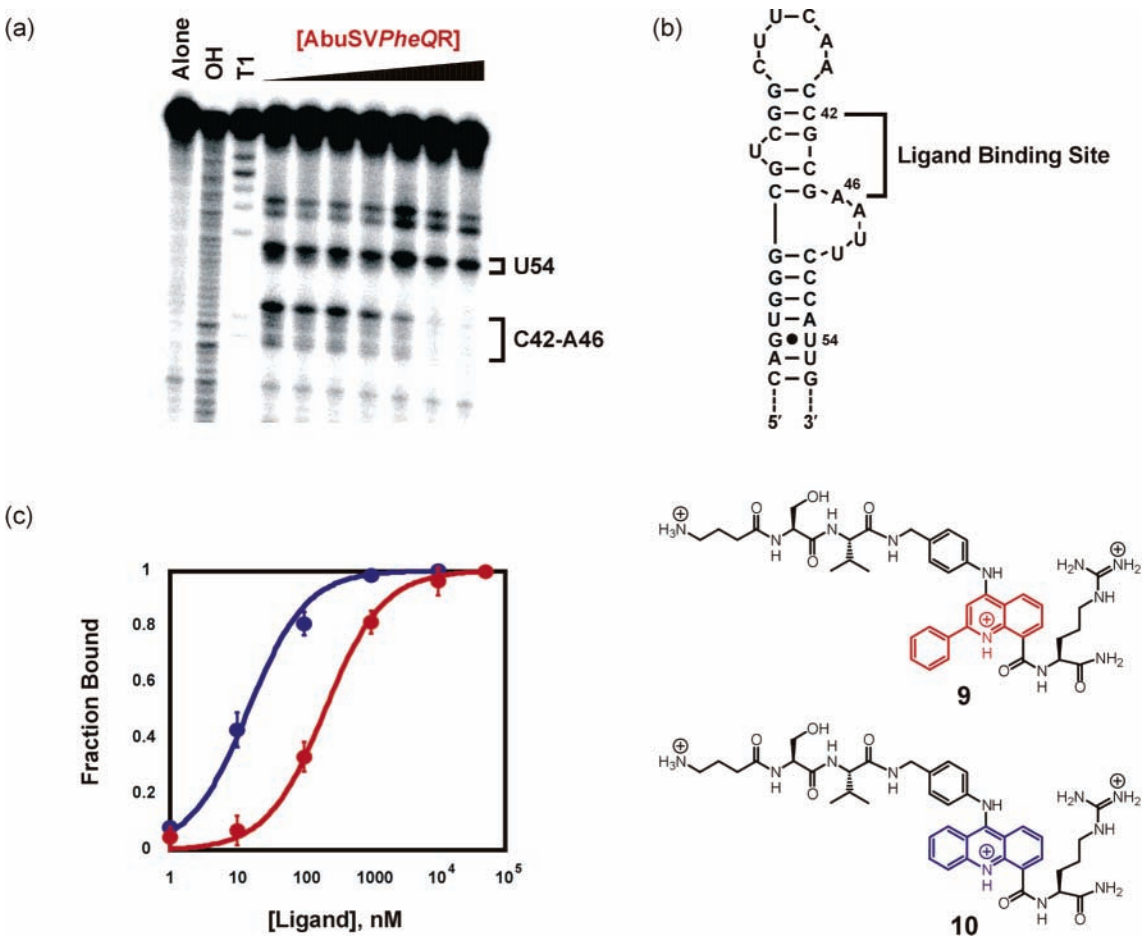

Figure 2. (a) Quantitative ribonuclease V1 footprinting with AbuSV*PheQ*R (**9**) and an in vitro selected RNA. Shown is a partial storage phosphor autoradiogram of a 10.5% denaturing polyacrylamide gel separating 5′-^{32}P-labeled RNA cleavage products. First three lanes, from left to right: Alone, RNA in buffer only; OH, alkaline hydrolysis; T1, RNase T1. The remaining lanes contain increasing concentrations of **9** (from left to right, 0, 0.001, 0.01, 0.1, 1, 10, 50 μM) in the presence of RNase V1. The footprinted region (C42−A46) is bracketed, as well as the constant V1-dependent nucleotide (U54) used to normalize for differential loading of the gel. (b) Secondary structure of nucleotides C22−G56 of the RNA aptamer as predicted by *mfold*.[20,21] The binding site for **9** is indicated. (c) Plot of fraction bound versus concentration of **9** (red) and **10**[22] (blue) as determined by RNase V1 footprinting.

preferentially to base-paired 5′-CpG-3′ sites in RNA flanked by non-Watson−Crick structural elements (Figure 2).[22] We determined the extent to which **9** bound to an in vitro selected RNA containing such a site using ribonuclease V1 footprinting (Figure 2a). Peptide **9** bound this stem-loop structure to give a footprint similar to that observed with **10**, indicating peptides **9** and **10** bind the same site on this RNA.[22] Quinoline peptide **9** binds with a K_D = 208 nM, whereas acridine peptide **10** bound with a K_D = 20 nM (Figure 2c). Interestingly, this difference in affinity is similar to that observed for a 2-phenylquinoline substitution for acridine in a known DNA intercalator.[15] Thus, the decreased binding affinity of peptide **9** compared to that of **10** likely arises from a decrease in efficacy of intercalation.

In summary, the synthesis of 2-phenylquinoline amino acids compatible with SPPS with Fmoc-protected amino acids has been accomplished. A peptide prepared with a 2-phenylquinoline amino acid binds an RNA stem-loop structure at the same site as the corresponding acridine-based threading peptide with an approximately 10-fold lower affinity. The strategy developed is applicable to the preparation of a variety of 2-substituted quinoline amino acids for systematic alteration of intercalator affinity in threading intercalator peptides. Since quinolones represent an important class of antibacterials, these compounds (conjugates and/or libraries) may also be useful in the discovery of new antibacterial agents.

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Supporting Information Available: Experimental procedures and NMR and mass spectral data for compounds listed in Scheme 1 and general procedure for the synthesis of **9** and its biochemical analysis, including RNA synthesis and labeling and ribonuclease footprining. This material is available free of charge via the Internet at http://pubs.acs.org.

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