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SYNTHESIS AND BINDING STUDIES OF A 1-ALKYL-3,6-DIAMINO-4-QUINOLONE BASED RECEPTOR FOR *N*-ACYLATED DIPEPTIDES

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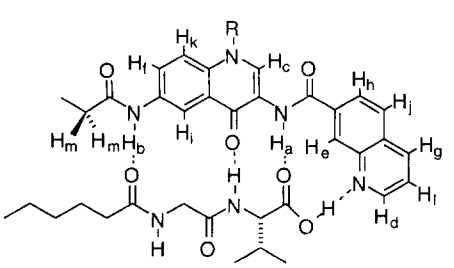
Abstract: The synthesis and binding properties of a semirigid host for *N*-acyldipeptide carboxylic acids is presented. The design is based on the rigidification of a peptide strand, coupled to the use of a substituted quinoline as a hydrogen bond acceptor for the proton of a carboxylic acid.

In the past few years there has been intense interest in the design of receptors for small peptide substrates.[1-4] A major source of inspiration is the mechanism of action of the antibiotic vancomycin, which binds to the terminal D-Ala-D-Ala sequence of bacterial cell wall peptidoglycans.[5] In the past, we have shown that carefully positioned hydrogen bonding groups can be used to recognize carboxylic acid and acylamino acid substrates.[6] In a further development of this strategy molecule **1** (Figure 1) was designed to complement the hydrogen bond donor and acceptor pattern found in the target guest molecule *N*-(*N*-hexanoylglycyl)-L-valine (**2**). The 1-alkyl-3,6-diamino-4-quinolone moiety, a peptidomimetic, derives from rigidification of one-half of a β-sheet, as seen in Figure 2. Although related structures have been used elsewhere in intramolecular β-sheet/turn mimics,[7-10] this is the first time it has been used as part of a receptor for a separate molecule. Nowick has recently used intramolecular hydrogen bonding to rigidify a 3-aminobenzoic acid derivative to produce a molecule, analogous to the diaminoquinolone skeleton presented in this work, that was used as part of an intramolecular β-sheet mimic.[11] Additionally, we covalently attached quinoline to our peptidomimetic to provide a binding site for the terminal carboxylic acid. Molecular modeling studies[12] of the **1•2** complex support the viability of the design: all hydrogen bond distances in this complex fall between 1.8 and 2.0 Å, indicating good shape complementarity. The synthesis of **1a** is shown in Scheme 1.



1a: R = *n*-Pr
1b: R = Et

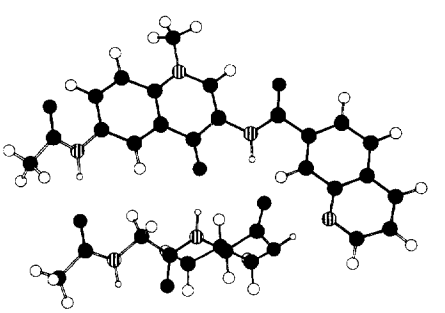


Figure 1. (left) Proposed complex of **1** and **2**; (right) Computer-generated minimized structure of the complex (MacroModel/Amber). Note that the alkyl groups have been shortened for clarity.

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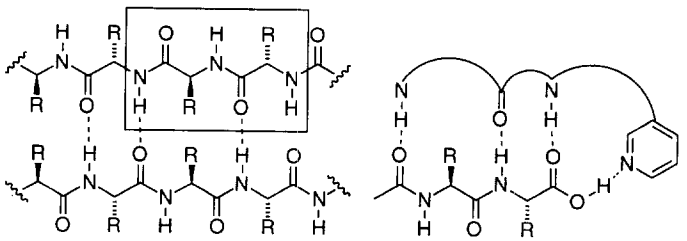
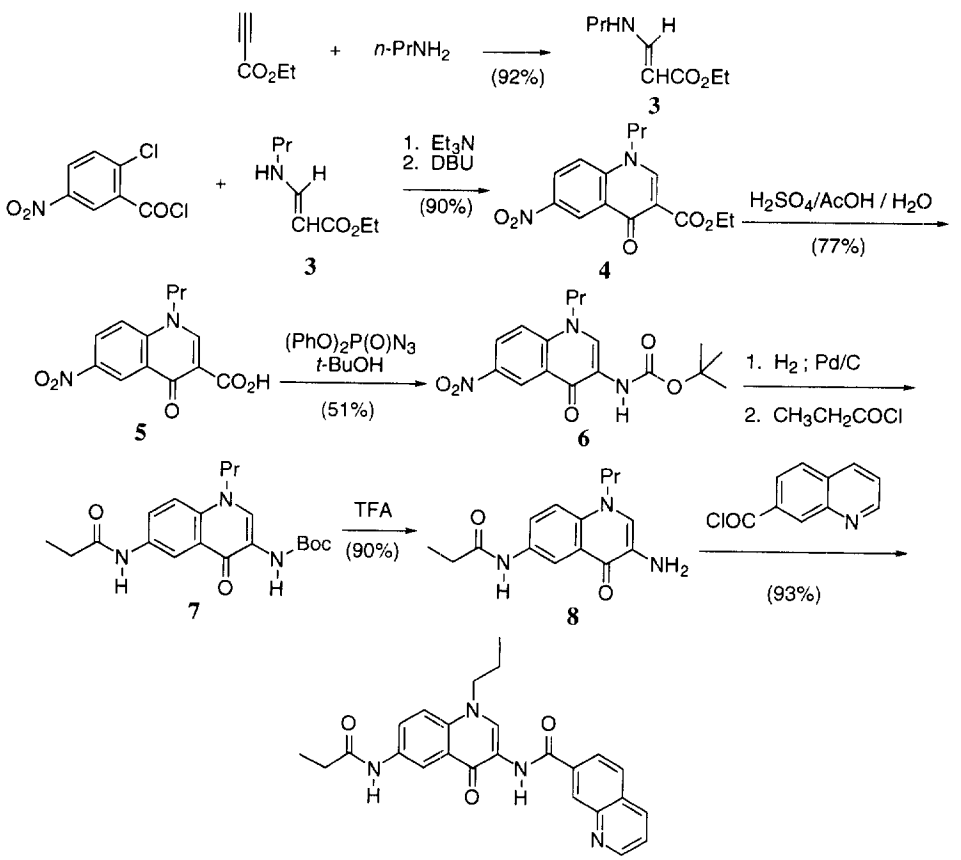


Figure 2. (left) Antiparallel β-sheet, indicating portion to be rigidified; (right) schematic of target complex.

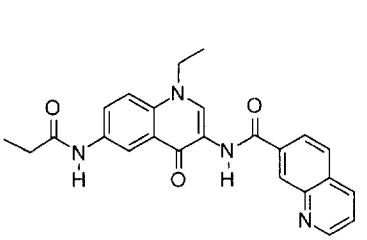


Scheme 1. Synthesis of host **1a**.

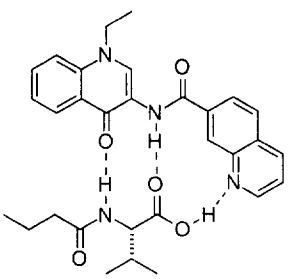
The synthesis of **1a** makes use of newer methods of quinolone synthesis.[13-15] Starting from commercially available ethyl propiolate and *n*-propyl amine, amino ester **3** was made as a mixture of *Z*-/*E*-isomers. Acylation with 2-chloro-5-nitrobenzoyl chloride followed by ring closure via an S_NAr displacement of the chloride afforded quinolone **4**, which was hydrolyzed in acid to give **5**. Use of diphenylphosphoryl azide in a Curtius reaction gave urethane **6**, an orthogonally protected diamine. Reduction of the 6-nitro group, acylation with propionyl chloride, removal of the Boc group with trifluoroacetic acid and acylation with quinoline-7-carbonyl chloride gave **1a** in nine steps (seven isolated compounds) in 27% overall yield. Despite the number of

steps involved, the synthesis of **1a** was straightforward as all compounds except **3**, **8**, and **1a** were purified by recrystallization;[16] **3** was purified by a simple distillation and **8** and **1a** were purified by silica gel chromatography. The quinoline-7-carbonyl chloride used was synthesized from commercially available 7-methylquinoline first by recrystallization of the dichromate salt from water followed by oxidation of this dichromate complex with additional dichromate in sulfuric acid. This acid was treated with thionyl chloride to give the acid chloride.

An alternative synthetic pathway to two related host molecules, **1b** and **9** (Figure 3), was also developed. Host **1b** (the *N*-ethyl analog of host **1a**) was formed by initially reacting methazonic acid with anthranilic acid to give 2-(2-nitroethylideneamino)-benzoic acid, which upon treatment with sodium acetate in acetic anhydride formed 3-nitro-quinolin-4-ol.[17] Alkylation with ethyl iodide gave 1-ethyl-3-nitro-4-quinolone. Tin(II) chloride reduction was followed by acylation with acetic anhydride to give 3-acetamido-1-ethyl-4-quinolone, which was nitrated in the 6- position.[18] Reduction (hydrazine/$FeCl_3$/carbon)[19] and acid hydrolysis gave 3,6-diamino-1-ethyl-4-quinolone as the dihydrochloride salt. This compound was acylated with quinoline-7-carbonyl chloride at the 3-amino group in 24% yield. Acylation of the 6-amino group with propionyl chloride afforded host **1b**. Host **9** was synthesized by acylation of 3-amino-1-ethyl-4-quinolone with quinoline-7-carbonyl chloride.



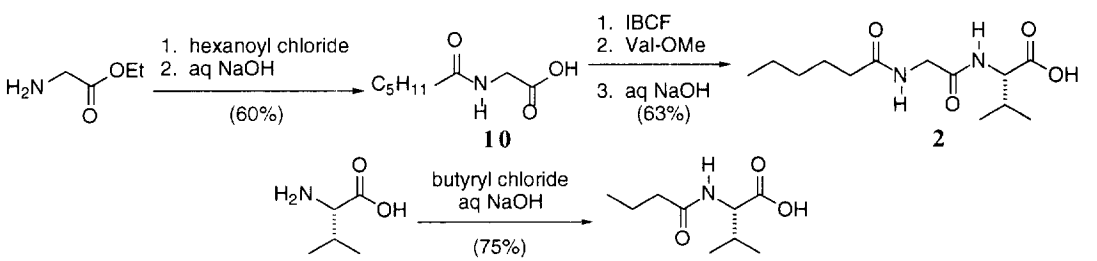
1b



9•11

Figure 3. Additional hosts **1b** and **9**.

Guest molecules **2** and **11** were synthesized as shown in Scheme 2. Acylation of glycine ethyl ester with hexanoyl chloride followed by saponification gave acid **10**, which was reacted with isobutyl chloroformate then coupled with L-valine methyl ester. Saponification of this compound gave **2**. *N*-Butyryl-L-valine was produced under Schotten-Baumann conditions from L-valine to give **11**.



10 **2** **11**

Scheme 2. Synthesis of guest molecules **2** and **11**.

Association constants were determined by a standard NMR titration method in which increments of a concentrated solution of guest in a known concentration of host were added to a solution of host at that same concentration and the spectra recorded. Nonlinear regression analysis of the binding data yielded association constants as presented in Table 1.[20] It can be seen that very strong binding takes place between **1b** and *N*-(*N*-hexanoylglycyl)-L-valine (**2**) in $CDCl_3$. Deletion of one amide bond from either the guest molecule (**1a•11**) or the host (**9•11**) results in complexes with one fewer hydrogen bonds and a substantial decrease in binding was seen. Significant binding was also seen in 1% CD_3OD in $CDCl_3$ for the **1a•2** complex; the association constant between **1a** and **11** was minimal in this same system. These results are consistent with the structures of the complexes as proposed; i.e., a bidentate interaction to the terminal carboxylic acid plus two hydrogen bonds to the peptide backbone as shown in Figure 1.

Table 1. Association Constants Between Hosts **1a**, **1b**, and **9** and Guests **2** and **11** in Various Solvents.

Host	Guest	Solvent	Temp. (K)	K_a[a] ($dm^3\ mol^{-1}$)	$-\Delta G$ (kcal mol^{-1})
1b	**2**	$CDCl_3$	297	1.6×10^4	5.7
1a	**11**	$CDCl_3$	293	2.9×10^2	3.3
9	**11**	$CDCl_3$	298	1.7×10^2	3.0
1a	**2**	1% CD_3OD in $CDCl_3$	294	8.4×10^2	3.9
1a	**11**	1% CD_3OD in $CDCl_3$	292	< 50	< 2.3

[a] Errors were estimated at ± 20%.

Examination of the complex-induced shifts provides insight into the binding event. Table 2 gives the chemical shift data for the amide protons of the hosts for the titrations performed in $CDCl_3$. In all cases H_a is significantly downfield at the start of the titration. (All labels refer to the protons as indicated in Figure 1.) We attribute this to deshielding from the carbonyl of the quinolone; i.e., a strong intramolecular hydrogen bond is present. This severely limits additional deshielding that can occur upon hydrogen bond formation to the guest, resulting in a small $\Delta\delta$. On the other hand, no such intramolecular hydrogen bond exists for H_b and substantial movement is seen in the titrations of **1** with **2** or **11**. The movement of H_b in the case of **1a•11** can be attributed to the conjugated nature of the host. The shifts of some of the C-H protons for the titration of **1a** with **2** in 1% CD_3OD in $CDCl_3$ are plotted in Figure 4, with results in Table 3.

Table 2. Calculated K_a, δ_0 and $\Delta\delta$ Values for the Amide Protons in the Host•Guest Complexes Shown ($CDCl_3$; K_a units of $dm^3\ mol^{-1}$; δ units of ppm)

complex	K_a	H_a		H_b	
		δ_0	$\Delta\delta$	δ_0	$\Delta\delta$
1b•2	16000	ca. 9.43	ca. 0.16	7.50	2.54
1a•11	290	9.43	0.06	7.51	1.29
9•11	170	9.49	0.09		

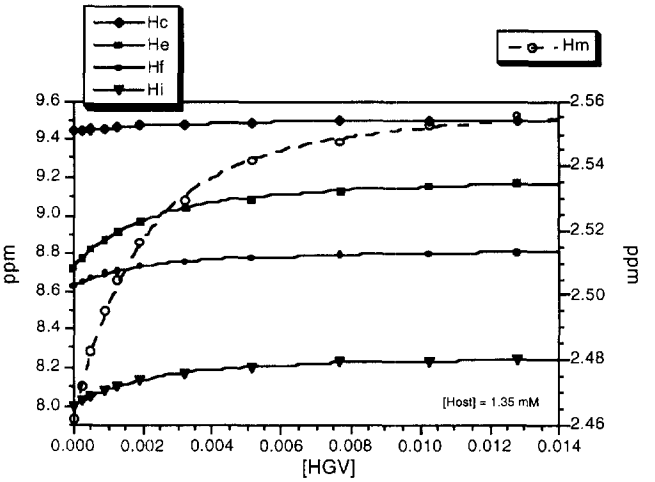


Figure 4. Binding curves for some C-H protons from the titration of **1a** with **2** in 1% CD_3OD in $CDCl_3$.

Table 3. Calculated K_a, δ_0 and $\Delta\delta$ Values for the C-H Protons in the **1a•2** Complex (1% CD_3OD in $CDCl_3$; K_a units of $dm^3\ mol^{-1}$; δ units of ppm)

	H_c		H_e		H_f		H_i		H_m	
K_a	δ_0	$\Delta\delta$	δ_0	$\Delta\delta$	δ_0	$\Delta\delta$	δ_0	$\Delta\delta$	δ_0	$\Delta\delta$
840	9.44	0.08	8.73	0.48	8.63	0.19	8.00	0.27	2.46	0.10

The magnitude of the complex-induced shifts for the C-H protons supports binding as envisioned. As with H_a, H_c is deshielded by an intramolecular interaction in the pure host, this time to the quinoline-7-carbonyl. This relative orientation of the quinoline to the quinolone is expected for both electronic and steric reasons: (pseudo)planarity allows for conjugation throughout the system. Rotation of the quinolone-3N bond by 180° would result in significant dipole-dipole interactions between the quinolone carbonyl and the quinoline carbonyl; furthermore, this would disrupt the intramolecular bond already mentioned. A *cis* amide bond between the two systems is precluded on steric grounds. The 6-propionamido group does not have these restrictions and presumably there is an equilibrium of the *trans* amide between conformations where the carbonyl is near H_f or near H_i. Complexation to guest **2** would shift this equilibrium towards the conformation shown, resulting in deshielding of H_f by the carbonyl in an intramolecular fashion and H_m and H_i by the carbonyl of the guest. Most significantly, H_e shifts downfield by almost 0.5 ppm, which is attributed to the close proximity of the carbonyl of the carboxylic acid in the host•guest complex.

Additional evidence supports the **1•2** complex as shown. A Job's Plot[21] was performed on **1a** and **2** in 1% CD_3OD in $CDCl_3$ and the data indicate 1:1 complex formation. Although cocrystals of **1a•2** could be reproducibly grown they were not of X-ray quality.[22] A solution of these crystals in $CDCl_3$ clearly showed the occurrence of two overlapping *N*-methylene triplets in **1a** indicating chemical non-equivalence of the complex-induced diastereotopic protons. The integration from the NMR of these cocrystals confirms the 1:1 stoichiometry. Lastly, an NOE difference experiment was done on this complex in $CDCl_3$ and an NOE effect

was seen between the glycyl protons and H_i of the quinolone system as well as the β-methylene of the hexanoyl group in **2** and H_m of **1a**.

A straightforward synthesis of a semi-rigid *N*-acyldipeptide binding host has been presented. This design made use of 1-alkyl-3,6-diamino-4-quinolone as a rigid peptide analog and evidence was given demonstrating the viability of this design. Incorporation of this moiety into other systems has been undertaken and details will be reported elsewhere.

Acknowledgment

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