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# Synthesis and binding studies of carboxylate binding pocket analogs of vancomycin

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**Abstract**

Synthetic receptors based on the carboxylate binding pocket of vancomycin were synthesized. Using a UV binding assay, affinities of various cell wall related carboxylates were measured in organic solvents. Affinities in the $10^3$–$10^4$ $M^{-1}$ range were determined as well as almost equal affinities for acylated alanine and lactate. Introduction of additional hydrogen bonding donors or positive charges into the receptors led to increased affinities for the carboxylates. © 2000 Published by Elsevier Science Ltd.

In the battle against pathogenic bacteria vancomycin is an important player as the drug of last resort against Gram-positive pathogens such as methicillin-resistant *S. aureus*. Considering its clinical importance it is worrisome that vancomycin-resistant *S. aureus* have emerged[1,2] and are likely to spread. In susceptible bacteria the drug binds to the D-Ala-D-Ala dipeptide sequence of the peptidoglycan cell-wall precursors and this prevents proper cell-wall construction, resulting in bacterial lysis. A common pattern with resistant bacteria is the occurrence of weakened binding due to replacement of the terminal D-Ala with a D-lactate residue, which constitutes a net replacement of an NH with an oxygen. This subtle replacement turns an attractive hydrogen bond into a dipolar repulsive interaction and results in a 1000-fold lower affinity.[3,4] Despite major efforts towards the synthesis of vancomycin[5] and vancomycin derivatization,[6] the minimal structural features needed for binding and selectivity are not known. Many compounds which represent part of the vancomycin structure have been prepared over the years[7] but their binding properties have not been systematically studied.[8] Considerable effort has also been devoted to creating synthetic receptors[9] for various targets including D-Ala-D-Ala and related peptides.[10] These have been highly instructive with respect to molecular recognition phenomena but the creation of selective high-affinity receptors for cell-wall peptides still remains an important goal. This report describes synthetic receptors that mimic part of the vancomycin structure and their binding to acylated alanine and lactate.

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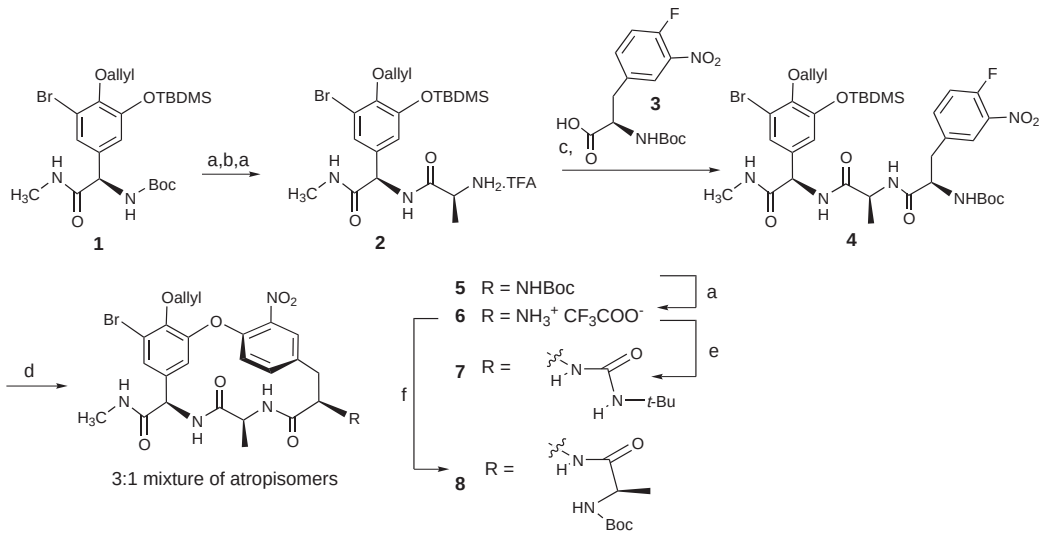
The syntheses started with **1**[11] derived from D-*p*-hydroxyphenylglycine. The amino group of **1** was deprotected with TFA and coupled to Boc-protected alanine by means of the coupling reagent HATU, after which the Boc group was removed, again with TFA to give **2**. The dipeptide was coupled to *N*-Boc-4-fluoro-3-nitro phenyl alanine (**3**) with HATU, to give tripeptide **4**. Amino acid **3** was obtained via diastereoselective alkylation of Schöllkopf's chiral bislactim ether.[12] Tripeptide **4** was cyclized using cesium fluoride in dimethylformamide. This reagent not only deprotects the silyl protected aromatic hydroxyl group but is also basic enough to act as a base in the $S_NAr$ macrocyclization.[13] The cyclization results in an inseparable 3:1 mixture of two atropisomers (**5**) with respect to the position of the nitro group. All further reactions and binding studies were conducted with this mixture of atropisomers. Deprotection of **5** by TFA resulted in the ammonium receptor **6**, which was then further functionalized. Reaction of **6** with *tert*-butyl isocyanate resulted in the urea compound **7**. Coupling of **6** to Boc-D-Ala led to receptor **8** (Scheme 1).




Scheme 1. *Reagents and conditions*: (a) TFA/$CH_2Cl_2$ 1:2, 2 h, quant.; (b) *N*-Boc-Ala, HATU, N$^i$Pr$_2$Et, $CH_2Cl_2$, DMF, 14 h, 51%; (c) HATU, N$^i$Pr$_2$Et, $CH_2Cl_2$, DMF, 14 h, 76%; (d) CsF, DMF, 14 h, 85%; (e) *tert*-butyl isocyanate, N$^i$Pr$_2$Et, $CH_2Cl_2$, 14 h, 71%; (f) D-Boc-Ala, HATU, N$^i$Pr$_2$Et, $CH_2Cl_2$, DMF, 14 h, 99%

Indications that the prepared molecules were indeed capable of binding peptidic cell-wall precursor ligands came from NMR spectroscopy. Addition of the tetra-*n*-butyl salt of *N*-Ac-D-Ala (0.7–8 mM) to a solution of receptor **7** in $CD_3CN$ (0.7 mM) resulted in downfield shifts of at least two of the NH resonances to 8.5 and 9.0 ppm.[14] These NH resonances were broad in the spectrum of the free receptor and located between 6.6 and 7.3 ppm. Furthermore, upfield and downfield shifts of all the aromatic protons were observed of ca. 0.1 ppm.[15] In organic media the association is mostly based on hydrogen bonding. Hydrophobic effects, which contribute significantly to vancomycin's binding free energy in water, are not addressed. However, since hydrogen bonding is of great importance for biological activity, or the lack thereof (see D-lactate replacement of D-Ala mentioned above), studies in apolar media may provide valuable insights into the nature of the receptor–ligand interactions and may suggest solutions for their optimization.

UV spectroscopy proved to be valuable for the evaluation of the binding strength of the receptors. Titration of a dilute solution of **5** ($1\times10^{-4}$ M in $CHCl_3$) with a solution of the tetra-*n*-butyl ammonium salt of *N*-Ac-D-Ala (while maintaining a constant receptor concentration), resulted in an increased absorption around 270–280 nm (Fig. 1). This increase could be fitted to a 1:1 binding isotherm and indicated an association constant of $1.9\times10^4$ $M^{-1}$, or 5.8 kcal $mol^{-1}$ of binding free energy. Very similar observations were made in the more polar solvent $CH_3CN$, although the affinity was somewhat lower and the absorbance differences upon complexation somewhat smaller ($\Delta$Abs. = 0.03–0.07 for all systems studied here at 270 or 280 nm).

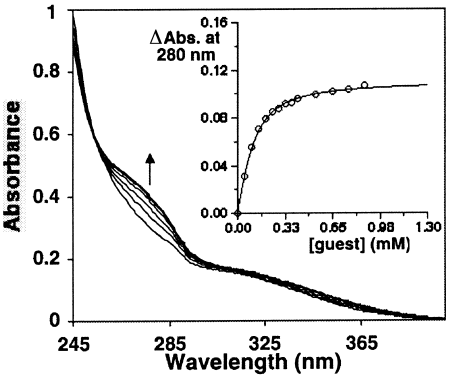



Figure 1. Shifts in the UV spectrum upon addition of the carboxylate of *N*-Ac-D-Ala in $CHCl_3$ to **5** [0.1 mM]. Inset: Binding curve of a titration experiment based on absorbance changes at 280 nm, association constant $K_a = 1.9\times10^4$ $M^{-1}$

In $CH_3CN$ several titrations were run and the results are summarized in Table 1.[16] These titrations indicated that the affinity of **5** for *N*-Ac-L-Ala (entry 2) was only marginally weaker than that for the D-enantiomer (entry 1). Vancomycin itself shows significant preference for D-Ala-D-Ala over its enantiomer,[17] which is either caused by hydrophobic effects in water, and/or by interactions which also involve the second alanine residue. Interestingly, in our titrations, changing the alanine residue for a lactate (entry 3) results in only a small drop in

Table 1
Binding affinities of receptor **5**–**8** [0.1 mM] to the tetra-*n*-butyl ammonium salts of various guests, expressed as association constants ($K_a$, $M^{-1}$) and free energies (kcal $mol^{-1}$, 1 cal = 4.184 J), determined by UV titrations monitoring the absorbance at 270 nm, at 296 K in $CH_3CN$

| Entry | Receptor | Guest | $K_a$ ($M^{-1}$) | $-\Delta G$ (kcal $mol^{-1}$)[a] |
|---|---|---|---|---|
| 1 | **5** | *N*-Ac-D-Ala | $9.7\times10^3$ | 5.4 |
| 2 | **5** | *N*-Ac-L-Ala | $6.9\times10^3$ | 5.2 |
| 3 | **5** | Ac-L-Lac | $5.8\times10^3$ | 5.1 |
| 4 | **5** | $OAc^-$ | $3.8\times10^2$ | 3.5 |
| 5 | **6** | *N*-Ac-D-Ala | $3.2\times10^4$ | 6.1 |
| 6 | **7** | *N*-Ac-D-Ala | $3.7\times10^4$ | 6.2 |
| 7 | **8** | *N*-Ac-D-Ala | $2.3\times10^4$ | 5.9 |

[a] Estimated error in $\Delta G$: $\pm0.2$ kcal $mol^{-1}$.

affinity. However, acetate (entry 4) binds much more weakly to receptor **5** with a $K_a$ of 380 $M^{-1}$. These results are in contrast to binding studies with vancomycin in aqueous buffer where acetate and Ac-D-Lac bind similarly weakly (30 and 40 $M^{-1}$)[18] and *N*-Ac-D-Ala binds an order of magnitude stronger, presumably due to the extra hydrogen bond that can be formed to its NH. Our observations may be due to the following: the system has the possibility to present either a hydrogen bond donor or an acceptor, depending on the nature of the guest, by rotating the CONHMe group. This would explain the similar affinities for the alanine and lactate containing guests and the drop in affinity of acetate.[19]

In order to increase binding affinities, the carboxylate binding pocket of **5** was modified by introducing additional hydrogen bonding donors or positive charges.[20] Indeed, the ammonium compound **6** binds the carboxylate ligand significantly stronger (entry 5), presumably due to additional electrostatic interactions. Introduction of a urea functional group in **7** (entry 6), which constitutes an O to NH mutation, led to an increased binding free energy of 0.8 kcal $mol^{-1}$ compared to **5**. The protected alanine compound **8** (entry 7) also results in binding enhancement, possible due to additional interactions of the carboxylate ligand with the Boc NH.

In summary, vancomycin binding pocket model systems have been prepared that are capable of binding *N*-Ac-Ala carboxylates and acylated lactate. The use of organic solvents was required here to observe binding. However, it provides a magnifying glass for the important hydrogen bonds, which are weak in water, and led to affinities in the $10^3$–$10^4$ $M^{-1}$ range. For comparison, in aqueous solution, even vancomycin binds to acylated D-Ala and D-lactate only weakly ($K_a$ values are 300 and 40 $M^{-1}$, respectively).[18] With our receptors a relatively high affinity for acylated lactate versus alanine was measured. This interesting phenomenon is possibly due to the freely rotatable C-terminal CONHMe group and underscores the promise of creating vancomycin analogs with altered hydrogen bonding characteristics. Modification of receptor **5** led to increased affinities with the introduction of additional hydrogen bond donors or positive charges. This augurs well for a combinatorial approach of further structural optimization, which is currently in progress.

## Acknowledgements

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8. For a related system and an early exception see: Pant, N.; Hamilton, A. D. *J. Am. Chem. Soc.* **1988**, *110*, 2002. This system has affinities for simple carboxylates that are of the same order of magnitude ($K_a$ cyanoacetate 580 $M^{-1}$ in $CDCl_3$ versus $K_a$ of $OAc^-$ for **5** is 380 $M^{-1}$ in $CH_3CN$, Table 1, entry 4) as those measured here, but included a benzyl amine instead of the phenyl glycine unit and therefore lacked the additional hydrogen bonding group responsible for the high D-Ala affinity in the present system.
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15. Downfield NH shifts are also observed for the $NBu_4^+$ salts of $OAc^-$ and Ac-L-Lac and distinct variations are observed in the complexation induced shifts of the resonances of the receptor for each guest.
16. Both receptor and carboxylate were thoroughly dried under high vacuum. A 0.1 mM solution of the receptor was prepared in $CH_3CN$ and a carboxylate solution was prepared using the receptor solution. Carboxylate concentrations ranged from 2 to 40 mM depending on the affinity. In a double beam spectrophotometer spectra were taken after incremental additions (up to 400 μL in total) of the carboxylate solution to a receptor solution (1.0 mL) in a Teflon stoppered cuvette. The cuvette was briefly vortexed after each addition. A cuvette containing $CH_3CN$ was used in the reference beam. From the collected data the absorbance changes at 270 or 280 nm were fitted to a 1:1 binding isotherm using a nonlinear least squares regression in ASSOCIATE 1.6 (Peterson, B. K.; Ph.D. Dissertation, UCLA, 1994). The carboxylates do not absorb at this wavelength. Experiments were run in duplicate or triplicate and results were averaged using material from different synthetic batches. HPLC grade $CH_3CN$ (Biosolve Ltd., water <0.02%) was used and experiments were run at 296 K. In this study atropisomeric mixtures were used, which will similarly be obtained in our planned combinatorial synthesis and screening on solid phase. Although this fact does not seem to preclude structure–affinity studies we will pursue binding studies of single isomers as well as detailed studies into the binding mode.
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