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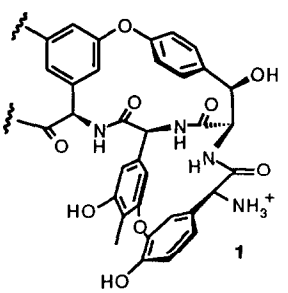
# SYNTHETIC ANALOGS OF THE RISTOCETIN BINDING SITE: NEUTRAL, MULTIDENTATE RECEPTORS FOR CARBOXYLATE RECOGNITION

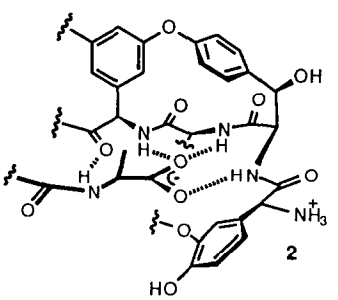
Jeffrey S. Albert and Andrew D. Hamilton*
Department of Chemistry, University of Pittsburgh
Pittsburgh, PA 15260

**Abstract**: A new family of receptors for carboxylates has been developed based on the multidentate recognition strategy of ristocetin. Particularly strong binding is seen with receptors that employ hydroxyl binding sites.

The strong and selective binding of amino acid carboxylates by the antibiotic ristocetin has been the subject of much recent discussion.[1] The key carboxylate binding pocket (**1**) is composed of three backbone amide-NH groups which form hydrogen bonds to the carboxylate oxygens (**2**). Remarkably, the nearby N-terminal ammonium group plays only a minor role in the complexation and can be removed with little change in binding affinity.[2] Our interest in the design of synthetic analogs of the ristocetin antibiotics[3] prompted us to search for neutral receptors that use a similar strategy of carboxylate recognition via multiple hydrogen bonding interactions (shown schematically in **3**).




**1**

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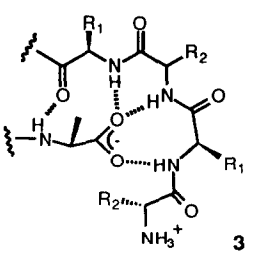
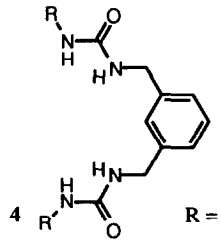
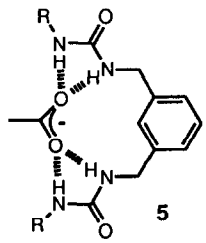



**3**

An initial approach involved simple modification of our work on the design of bis-urea receptors for dicarboxylates.[4,5] Reaction of 1, 3-bis(aminomethyl)benzene (in place of the 1, 4-derivative[4]) with n-butyl isocyanate gave bis-urea **4** (85% yield) in which four hydrogen bond donors are positioned to interact with the



4 R = n-butyl



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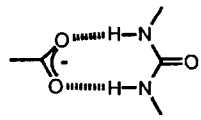
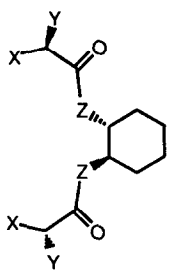


6

two carboxylate oxygens, as in **5**. The binding properties of **4** were conveniently followed by $^1$H NMR spectroscopy. Titration of **4** in $CD_3CN$ with tetrabutylammonium acetate (TBAA) gave large downfield shifts of the urea NH resonances (> 1.8 ppm) consistent with the formation of a hydrogen bonded complex in solution. Non-linear regression analysis of the binding curve[6] gave an association constant of 2,240 $M^{-1}$. That both ureas are participating in binding was confirmed by a Job's plot which showed a maximum at mole ratio 0.5 (consistent with 1:1 stoichiometry) and by the ten-fold weaker binding of acetate by 1, 3-dimethyl urea (1, 3-DMU, Table 1). Remarkably, the downfield shift at saturation of the urea-NH resonance is larger (3.3 ppm) in the weaker complex **6** than in **5** (1.8 ppm). This is consistent with two different geometries for the carboxylate/urea complexes; an eight membered ring bidentate interaction in **6** and two six membered H-bonded rings in **5**. In **6** only one urea-NH group binds to each acetate oxygen leading to large downfield shifts but weak binding involving just two hydrogen bonds. However, in **5** the basicity of each acetate oxygen is dissipated through two NH groups hence the NMR shifts are smaller but the binding by four, albeit weaker, hydrogen bonds is stronger. The different electrostatic character of the hydrogen bonds also accounts for the relatively small, ten-fold advantage of bis-urea vs. monourea binding.

An alternative approach to carboxylate recognition involves separating the hydrogen bond donor sites in the receptor by two carbon atoms (as opposed to one in **5**), as seen in the ristocetin/D-Ala complex **3**. Such an arrangement can readily be constructed from the reaction of (1*R*, 2*R*)-trans-1, 2-diaminocyclohexane (**7**) with an



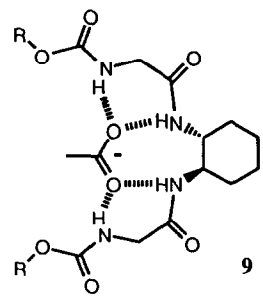
**8**. X=NHCbz, Y=H, Z=NH

**10**. X=NHCbz, Y=$CH_2OH$, Z=NH

**11**. X=NHCbz, Y=$CH_3$, Z=NH

**12**. X=NPhth, Y=$CH_2OH$, Z=NH

**13**. X=NHCbz, Y=$CH_2OH$, Z=O



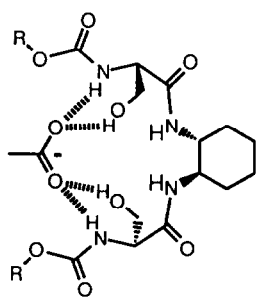
**9**

| Receptor | Solvent | $K_a$ ($M^{-1}$) | Receptor | Solvent | $K_a$ ($M^{-1}$) |
|---|---|---|---|---|---|
| **4** | $CD_3CN$ | $2.2 \times 10^3$ | **10** | $d_6$-DMSO | 89 |
| **1,3-DMU** | $CD_3CN$ | $2.7 \times 10^2$ | **11** | $CD_3CN$ | $3.4 \times 10^2$ |
| **8** | $CD_3CN$ | $2.1 \times 10^2$ | **12** | $CD_3CN$ | $1.4 \times 10^3$ |
| **10** | $CD_3CN$ | $2.7 \times 10^5$ | **13** | $CD_3CN$ | $1.0 \times 10^4$ |

**Table 1.** Association Constants for Tetrabutylammonium Acetate Binding to Multidentate Receptors.[6]

N-protected amino acid. For example, the CDI-mediated coupling of **7** and N-carbobenzyloxy-glycine gave receptor **8**, in which four amide-NH groups are positioned to bind to acetate as in **9**. Addition of TBAA to a $CD_3CN$ solution of **8** caused large downfield shifts of the amide-NH and urethane-NH resonances (1.31 and 1.84 ppm at saturation, respectively) consistent with a complex structure of type **9**. However, the arrangement of binding sites is not optimal and only weak association is seen (Table 1).

A dramatic increase in binding affinity was observed when the amino acid component was changed to L-serine, as in receptor **10**. The incorporation of two hydroxyl groups into the binding site leads to a 1000-fold increase in the association constant over the glycine (**8**) or L-alanine (**11**) derivatives. The role of the urethane-NH and amide-NH groups also appears to change. In the presence of excess of TBAA the urethane-NH resonance of **10** shifts significantly downfield (2.03 ppm) while the amide-NH is little affected (0.44 ppm).[7] This is consistent with a complex of structure **14** in which the hydroxyl and urethane-NH groups provide the primary hydrogen bonding contact to the acetate with only a weak interaction from the amide-NH. Further support for this structure comes from a systematic deletion of the different binding sites in **10**. Receptors **11**, **12**, and **13** are missing the hydroxyl, urethane-NH and amide-NH groups, respectively. The largest drops in $K_a$ are seen with **11** (800-fold) and **12** (200-fold) confirming the important role of the serine hydroxyl and urethane



**14**

groups in binding. Furthermore, a Job's plot of **10**:TBAA confirmed a 1:1 stoichiometry for the complex and the cyclohexylamide of N-Cbz-serine (corresponding to half of receptor **10**) showed a much reduced binding constant ($4.6 \times 10^3\ M^{-1}$), consistent with the participation of both amino acids in acetate binding in **14**.

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6. Using the Hostest-II program, Wilcox, C. S. Frontiers in Supramolecular Organic Chemistry and Photochemistry, Schneider, H. J.; Dürr, H., Eds.; VCH: Weinheim, 1990. Errors were estimated to be ±40% for the very strong complex between **10** and TBAA and ±10% for all other complexes.
7. The hydroxyl protons were significantly broadened and could not be accurately followed in the titrations.

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