Selective Recognition of NH_4^+ over K^+ with Tripodal Oxazoline Receptors

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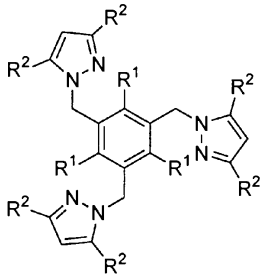
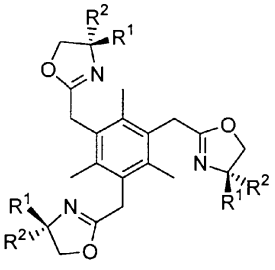
Benzene-based tripodal tris(oxazolines) are found to be promising receptors for the selective recognition of NH_4^+ over K^+ with high binding affinities.

The selective recognition of NH_4^+ from monovalent metal ions, particularly K^+, has attracted considerable interest in clinical chemistry.[1] The urea content in the blood is measured for the diagnosis of kidney function, after converting it into NH_4^+ by a urease. In measuring NH_4^+ the interference from K^+ is a severe problem because both are closest in size. Nonactin and its congeners, natural macrotetrolide ionophores, have been commercially employed as the recognition component in enzyme-based ion-selective electrode methods. However, nonactin provides a slight preference for NH_4^+ over K^+ (selectivity < 10), and improvement of the selectivity has been a subject of considerable research interest.[2] Very recently, Chin et al. have applied benzene-based tripodal pyrazole receptors **1** developed by Steel and co-workers[3] to the selective recognition of NH_4^+ over K^+.[4] A dramatic enhancement in the NH_4^+/K^+-selectivity (~400) compared to that of nonactin was achieved with receptor **1b**. However, it was found that the pyrazole receptors exhibited decreased detection sensitivity compared to nonactin.

Since pyrazole itself is weakly basic (pK_a = 2.5)[5] more basic ligands are expected to show enhanced binding affinities toward NH_4^+ (and also K^+). Among possible heterocyclic ligands, 2-oxazoline ligands were considered to be suitable candidates owing to their moderate basicity (pK_a ~5).[6] In addition, the oxazoline ligands have an advantage over the others in that they are readily synthesizable in various structural analogs.



1a R^1 = Me, R^2 = H
1b R^1 = Et, R^2 = Me



2a $R^1 = R^2$ = H
2b R^1 = Me, R^2 = H
2c R^1 = H, R^2 = Pr^i
2d $R^1 = R^2$ = Me

By diffusing diethyl ether into a mixture of tripodal oxazoline **2c**, synthesized from 1,3,5-tris(cyanomethyl)mesitylene and the corresponding amino alcohol,[7] and $NH_4^+PF_6^-$ in ethanol we were able to obtain single crystals that provided the corresponding host-guest complex by X-ray crystallography (Figure 1).[8]

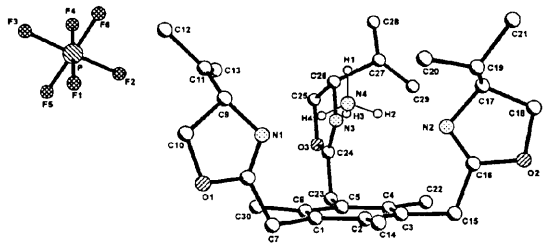


Figure 1. X-Ray structure of **2b**·$NH_4^+PF_6^-$ complex.

The oxazoline nitrogen is providing directional hydrogen bonds toward three ammonium NH bonds. The N_R-N_A lengths range from 2.78-3.06 Å, typical of hydrogen bonded N-H-N distances.[9] The values of N_R-N_A-N_R angles are in the range of 108–110°, hence little strain develops after the complex formation. The dihedral angles between the oxazoline nitrogen and the adjacent benzene carbon are in the range of -5.36–9.86°. Therefore, the crystal structure ideally matches between NH_4^+ and the receptor. The counter anion (PF_6^-) resides outside the complex with no apparent interaction. Overall, the ammonium complex of oxazoline **2c** maintains C_3-symmetry. This binding mode is also maintained in solution. A ^1H NMR study for a 1 : 1 complex of **2c** and NH_4^+ in CD_3OD-$CDCl_3$ (3 :2) at 25 °C shows an expected symmetrical structure with significant shifts for all the receptor protons. For example, up-field shifis for the benzylic (CH_3, 0.12; CH_2, 0.06 ppm) and isopropyl (CH_3, 0.10; CH, 0.06 ppm) protons are observable.

We evaluated association constants of NH_4^+ and K^+ with oxazoline **2a**–**2d** by the picrate extraction method developed by Lein and Cram.[10] Strong binding affinities as well as high ion-selectivities were observed, which are listed in Table 1.[11]

The association constants and their relative magnitude between NH_4^+ and K^+ were dependent on the oxazoline structure. The oxazoline **2b** exhibited the largest association constant toward NH_4^+ among tested receptors (K_a = 2.5x10^7 M^{-1}).

Table 1. Association constants (K_a x 10^{-3} M^{-1}) of ammonium and potassium ions (picrate salts) with receptors **2a-d** and nonactin, obtained by UV spectroscopy.[a]

	2a	2b	2c	2d	Nonactin
NH_4^+	5100 ± 600	25000 ± 2000	9400 ± 200	3900 ± 800	198000[b]
K^+	29.5 ± 0.1	57.2 ± 0.3	23.9 ± 0.4	57 ± 2	67100[c]
NH_4^+/K^+	173	437	393	68	3

[a]Measured in $CHCl_3$ at 25 °C. [b]A large deviation (± 70000) was observed. [c]Deviation: ± 2000.

Such a strong association has not been commonly observed except with pre-organized cryptate receptors.[2,12] The three directional hydrogen bonds by the oxazoline ligands and the presumed cation-π interactions[13] by the benzene ring are believed to provide such strong binding.[14] Compared to pyrazole receptor **1b**,[4] oxazoline receptor **2b** exhibits increased binding affinities to one order of magnitude towards both ions. To compare the binding affinity with nonactin under the same conditions, we carried out the picrate extraction experiment with nonactin. It was found that nonactin exhibited higher binding affinity in comparison with oxazoline **2b**. Nonactin also showed very high affinity toward K^+, hence poor selectivity between them (NH_4^+/K^+-selectivity = ~3) results, as already observed by others.[15] The highest NH_4^+/K^+-selectivity (~440) is also observed with oxazoline **2b**. In spite of considerable efforts to achieve high selectivity, so far such level of selectivity has only been observed with few receptors including the cage-like cryptate and the pyrazole receptors.[16] The origin of the NH_4^+/K^+-selectivity dependent on the substituents of the oxazolines has not been understood.

In summary, using novel benzene-based tripodal oxazoline receptors, we were able to selectively recognize NH_4^+ from interfering K^+. An impressive selectivity as well as high binding affinity is achieved, which indicate that the oxazolines are promising receptors for the selective recognition of NH_4^+ over K^+. A further study on the selective recognition of biologically more relevant amines with the oxazoline receptors is undergoing.

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6 The approximate pK_a value of 2-oxazoline (4,5-dihydrooxazole) may be inferred as follows: pK_a of 2-oxazoline ≈ pK_a (oxazole, 0.8) + [pK_a (2-imidazoline, 11.0) - pK_a (imidazole, 7.0)] ≈ 5. Other ligands such as imidazole, 2-imidazoline, and their derivatives can be considered. However, more basic nature of them may limit their practical usage at neutral pH.

7 For a review on the chemistry of oxazolines, see: T. G. Gant and A. I. Meyers, *Tetrahedron*, **50**, 2297 (1994). A detailed synthesis of tripodal oxazolines **2** will be reported elsewhere. Oxazolines **2b** and **2c** can be synthesized by the treatrnent of 1,3,5-tris(cyanomethyl)-2,4,6-trimethylbenzene with the corresponding amino alcohol (4.5 equiv) in the presence of $ZnCl_2$ (1.2 equiv) at reflux for 60 h under argon in 11% and 34% isolated yields, respectively. **2b**: mp 160–161 °C; $[\alpha]^{20}_D$ = +89.5 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.26 (dd, *J* = 9.1, 8.3 Hz, 3H), 4.13–4.06 (m, 3H), 3.71 (t, *J* = 7.6 Hz, 3H), 3.58 (s, 6H), 2.35 (s, 9H), 1.19 (d, *J* = 6.5 Hz, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.8, 136.1, 131.0, 74.3, 61.7, 30.5, 21.9, 17.4; MS (EI): *m/z* (%) 411 (M^+, 100); Anal. Calcd for $C_{24}H_{33}N_3O_3 \cdot 0.5H_2O$; C, 68.54; H, 8.15; N, 9.99. Found: C, 68.27; H, 7.89; N, 10.26. **2c**: mp 143–144 °C; $[\alpha]^{20}_D$ =-106.8 (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 4.19–4.13 (m, 3H), 3.94–3.88 (m, 6H), 3.73 (s, 6H), 2.38 (s, 9H), 1.80–1.74 (m, 3H), 0.86 (dd, *J* = 22.6, 6.8 Hz, 18H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.9, 136.1, 131.0, 72.2, 70.1, 32.7, 30.5, 19.2, 18.2, 17.5; MS (EI): *m/z* (%) 495 (M^+, 100); Anal. Calcd for $C_{30}H_{45}N_3O_3$; C, 72.69; H, 9.15; N, 8.48. Found: C, 72.86; H, 9.14; N 8.19.

8 Crystal data for **2c**·$NH_4PF_6 \cdot C_2H_5OH$: $C_{32}H_{55}F_6N_4O_4P$, M = 704.77, tetragonal, space group I4 (no. 79), *a* = 21.7947(2), *b* = 21.7947(2), *c* = 17.5167(3) Å, *U* = 8320.59(18) Å^3, Z = 8, *T*= 188(2) K, μ = 1.28 mm^{-1} D_c = 1.125 g/cm^3, F(000) = 3008, crystal size = 0.50 × 0.25 × 0.25 mm^3 Siemens SMART CCCD diffractometer, Mo-Kα radiation (λ= 0.71073 Å), 15815 reflections collected, 6023 independent reflections, R1 = 0.0779, wR2 = 0.2224 [I>2σ(I)], Rl = 0.0839, wR2 = 0.2330 (all data).

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11 The binding study is carried out as follows. A mixture of the picrate salt of NH_4^+ or K^+ (0.5 mL, 0.015 M in water) and the host (0.1 mL, 0.075 M in $CHCl_3$) in a centrifuge tube was equilibrated for an hour in a thermostat at 25 °C. After being kept for 1 h, the whole mixture was extracted by Vortex-Genie for 1 min and then centrifuged at 1500 rpm for 1 min. An aliquot of the $CHCl_3$ layer was measured and transferred by micro-syringe into a 5-mL volumetric flask and diluted to the mark with CH_3CN. For a more intensely colored layer 0.01-mL aliquot and for a less intensely colored layer 0.05-mL aliquot were used. The UV absorption of each 5-mL solution was measured at 380 nm. Determination of the binding constant was followed by the literature procedure.[10] The *R* value that represents the molar ratio of the guest to receptor **2b** in the organic phase was 0.67 for NH_4^+.

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16 The binding study by the picrate extraction method may not be directly compared with that of ion selective electrode experiments. An ISE study is undergoing in collaboration with a specialist.