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Synthesis of a Sensitive and Selective Potassium-Sensing Fluoroionophore

Richard D. Carpenter† and A. S. Verkman*

Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, California

alan.verkman@ucsf.edu

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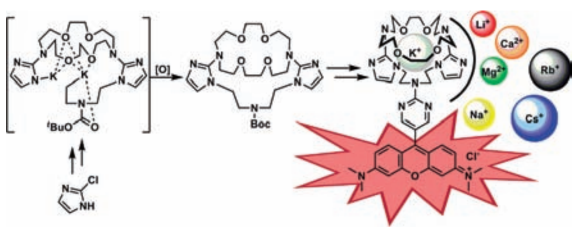
ABSTRACT



An efficient synthesis is reported that delivers in 5 steps and 52% overall yield a new structurally simplified fluorescent K^+ sensor with improved K^+ sensitivity and selectivity over existing K^+ sensors. The synthesis procedure utilizes a new template-directed oxidative C−N bond-forming macrocyclization reaction and reports new approaches to Pd(0), Sandmeyer-like and metal-free aminoarylations, as well as organotitanium additions to vinylogous sulfonates.

Efficient syntheses of complex, biomedically relevant compounds provide the impetus for novel reaction methodologies.[1] In the field of ion sensing, of great importance are fluorescent potassium (K^+) indicators that function in aqueous media and have good optical properties, high K^+ sensitivity and selectivity, pH insensitivity, and perhaps most importantly, a feasible synthetic route.[2] K^+ is a major analyte that plays a vital role in normal cell function and various diseases.[3] Our laboratory and others have made advances in K^+ biosensing, including the development of an aqueous-compatible sensor,[4a] an ionophore that is insensitive to pH in the range of 5−8,[4b] and a conjugated ionophore-chromophore system that by photoinduced electron transfer produced a 14-fold increase in fluorescence with increasing K^+.[4c,d] The reported synthetic methods for these sensors involve 12−16 steps (longest linear sequence 11−13 steps), which include two 21-membered macrocyclizations and a poorly yielding two- to three-step ionophore-chromophore union late in the synthesis.[4] Limited by these laborious routes, fine-tuning the K^+ sensitivity and selectivity of these sensors has not been done, nor have these sensors been widely available to biomedical scientists.

Herein is described a concise synthesis of structurally simplified and functionally superior K^+ sensor **1** via three interesting C−N bond-forming reactions and an efficient ionophore-chromophore C−C bond-forming reaction as shown in Figure 1. Highlights of this novel approach include a rapid microwave-mediated aminoarylation using a palladium-QuinaPhos catalyst, a template-directed macrocy-

† Presently at the Department of Biomedical Engineering, University of California, Davis, California.

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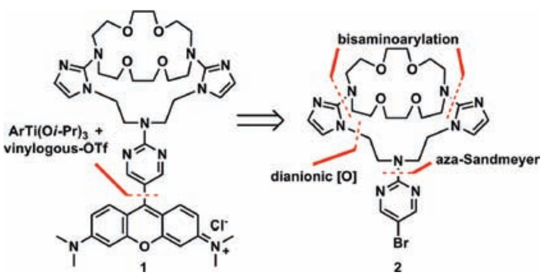

Figure 1. Structures and key disconnections of K^+ sensor **1** and ionophore bromide precursor **2**.

clization representing a conformationally unrestricted oxidative C−N bond-forming reaction, the first example of employing a secondary amine in a Sandmeyer-like reaction, a metal-free aminoarylation, and a one-step organotitanium-mediated ionophore-chromophore forming reaction. Each of these transformations not only is applied to the synthesis of **1** but also has been extended to demonstrate additional examples of their scope and utility. The efficient synthesis route developed here should enable the development and application of K^+ sensors for important biomedical applications, such as high-throughput screening/drug discovery,[5a] *in vivo* K^+ imaging,[5b] artificial receptors,[5c] fiberoptic K^+ sensing,[5d] and organellar K^+ measurement.

Our step-economical synthesis focused on simplifying and optimizing the K^+ binding motif as well as improving the ionophore-chromophore union. Previous ionophores have shown >30-fold selectivity for K^+ over biological ions such as Ca^{2+}, Mg^{2+}, and to a lesser extent, Na^+.[4] However, these sensors lack selectivity for the larger ions Cs^+ and Rb^+. We postulated that improved K^+ binding affinity and selectivity could be achieved by decreasing the size of the ionophore cage by four atoms and using Lewis basic bisimidazoles in lieu of tolyl moieties. Additionally, we envisioned that replacing the methoxyethoxyphenyl motif with pyrimidine would likely maintain or improve affinity and that ionophore bromide **2** as well as subsequent ionophore-chromophore conjugates would be more synthetically accessible than sensors derived from an ionophore aldehyde, the precursor used in prior syntheses.[4]

The function-oriented synthesis of **1** began with attempts toward a transition-metal-mediated bisaminoarylation as shown in Figure 2a. Unfortunately, established Cu(I)[6a] and Pd(0)[6b,c] methods were unsuccessful in our hands, as depicted in entries 2, 10, and 11. The halogen of the aryl halide, Pd(0) source and ligand, base, solvent, and heating element were all found to be critical components to obtain diimidazole **3**

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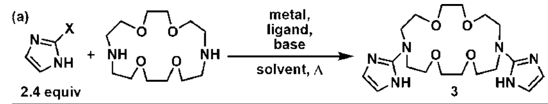
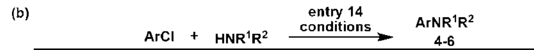
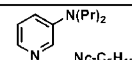
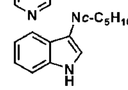
(a)



entry	X	metal	ligand	base	solvent	conversion (%)[a]	Λ element[b]
1	I	CuI	--	NaOH	DME	0	conventional
2[c]	Br	CuI	--	NaOH	dioxane	8	conventional
3	Cl	CuI	--	NaOH	dioxane	19	conventional
4	Cl	$Pd(PPh_3)_4$	--	LiHMDS	THF	23	conventional
5	Cl	$Pd(PPh_3)_4$	--	Cs_2CO_3	THF	29	conventional
6	Cl	$Pd(OAc)_2$	PPh_3	Cs_2CO_3	dioxane	28	conventional
7	Cl	$Pd(OAc)_2$	PPh_3	K_2CO_3	THF	44	conventional
8	Cl	$Pd(Cl)_2$	PPh_3	K_2CO_3	dioxane	51	conventional
9	Cl	$Pd(Cl)_2$	PPh_3	KHMDS	dioxane	35	conventional
10[d]	Cl	$Pd(Cl)_2$	JosiPhos	K_2CO_3	Ph_2O	43	conventional
11[e]	Cl	$Pd(Cl)_2$	BrettPhos	K_2CO_3	dioxane	49	conventional
12	Cl	$Pd(Cl)_2$	QuinaPhos	K_2CO_3	dioxane	86	conventional
13[f]	Cl	$Pd(Cl)_2$	QuinaPhos	K_2CO_3	dioxane	--	microwave
14	Cl	$Pd(Cl)_2$	QuinaPhos	K_2CO_3	DMF	93	microwave

(b)



compd	Ar	R^1	R^2	$ArNR^1R^2$	% yield
4	3-pyridyl	Pr	Pr	 N(Pr)$_2$	96
5	3-indolyl	c-C_5H_{10}		 Nc-C_5H_{10}	91
6	Ph	Me	Ph	$(Ph)_2NMe$	89

(c)

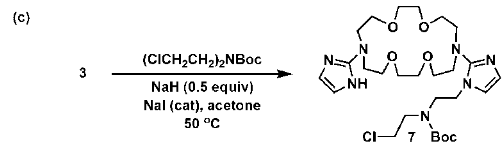


Figure 2. (a) Conditions shown in entry 14 used QuinaPhos and microwave irradiation (20 min) to afford **3** (93%). (b) Utility of this aminoarylation reaction is demonstrated in the syntheses of **4**−**6**. (c) Finkelstein alkylation of **3** to afford **7** (87%). Footnotes: [a]Determined by LCMS. The conversion % value in entry 14 also represents the isolated yield. [b]Conventionally heated reactions occurred in an oil bath at reflux for 18 h. Microwave reactions were heated to 95 °C for 20 min. Conditions used from [c]ref 6a, [d]ref 6b, [e]ref 6c. [f]Desired *T* could not be achieved.

in high yield without requiring protection of the imidazole NH. Entry 14 highlights the conditions found that feature 2-chloroimidizole as the aryl halide, QuinaPhos as a new ligand, and microwave irradiation as a heat source to afford **3** in 93% yield. Additionally, these aminoarylation conditions afforded tertiary arylamines **4**−**6** in excellent yields in 20 min using a variety of aryl chlorides with acyclic and cyclic secondary amines. This aminoarylation was then followed by a Finkelstein-mediated monoalkylation of diimidazole **3** with *tert*-butyl bis(2-chloroethyl)carbamate to provide alkyl chloride **7** (87%) as shown in Figure 2c.

With an efficient two-step route to **7**, effort was then focused on the synthesis of ionophore bromide **2** as shown in Figure 3. Unfortunately, various intramolecular *N*-alkylation attempts produced intermolecular or elimination products as shown in Chart 1 of Supporting Information. Inspired by recent dianionic oxidations on conformationally constrained systems,[7] we envisioned that deprotonating the N−H of **7** coupled with a metal−halogen exchange would

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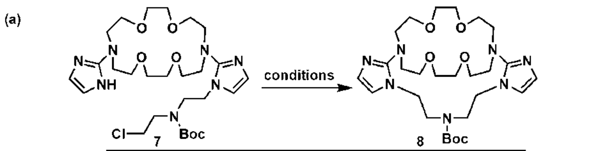
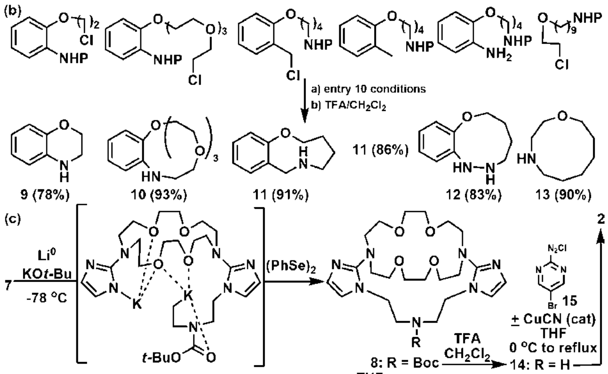


entry	metal/addiditves	oxidant	conversion[a]
1[b]	Li sticks	I_2	0%
2[c]	Na sticks	I_2	--
3[c]	K chunks	I_2	--
4[b]	Mg turnings	I_2	0%
5	Li sticks/KO*t*-Bu	I_2	84%
6	Mg turnings/KO*t*-Bu	I_2	45%
7[d]	Li sticks/KO*t*-Bu	TEMPO	0%
8[e]	Li sticks/KO*t*-Bu	CAN	0%
9[d]	Li sticks/KO*t*-Bu	DMP	0%
10	Li sticks/KO*t*-Bu	$(PhSe)_2$	92% (91%)



(d) R^1R^2NH + ClN_2Ar ± CuCN (cat) → (THF, 0 °C to reflux) R^1R^2NAr 16-22

R^1	R^2	Ar	compd	product	% yield[f]
n-Pr	*n*-Pr	*p*-$C_6H_4OCH_3$	16	(*p*-$C_6H_4OCH_3$)N(*n*-Pr)$_2$	73, 76
-$(CH_2)_4$-		*m*-$C_6H_4OCH_3$	17	(*m*-$C_6H_4OCH_3$)N[(-CH_2)$_4$-]	81, 75
-$(CH_2)_4$-		*o*-$C_6H_4OCH_3$	18	(*o*-$C_6H_4OCH_3$)N[(-CH_2)$_4$-]	65, 58
-$(CH_2CH_2)_2$O-		2-pyridyl	19	(2-pyridyl)N[-$(CH_2CH_2)_2$O-]	86, 88
n-Pr	*n*-Pr	Ph	20	PhN(*n*-Pr)$_2$	79, 74
-$(CH_2)_4$-		*p*-$C_6H_4CF_3$	21	(*p*-$C_6H_4CF_3$)N[(-CH_2)$_4$-]	89, 92
n-Pr	*n*-Pr	*p*-$C_6H_4CH_3$	22	(*p*-$C_6H_4CH_3$)N(*n*-Pr)$_2$	78, 72

Figure 3. (a) Conditions shown in entry 10 used Li sticks/KO*t*-Bu and $(PhSe)_2$ to afford **8** in an isolated yield of 91% yield. (b) Utility of this oxidative cyclization is demonstrated in the syntheses of cyclic secondary amines **9**−**13** (*P* = Boc). (c) Synthesis of ionophore **2** featuring an oxidative C−N bond-forming cyclization and either a Sandmeyer-like reaction (+CuOAc) or a metal-free aminoarylation (−CuOAc) with a secondary amine. (d) Utility of these aminoarylation conditions used in Figure 2c is demonstrated in the synthesis of tertiary arylamines **16**−**22**. Footnotes: [a]Determined by LCMS. Isolated yield shown in parentheses. [b]Major product was a dimer. [c]Starting material recovered. [d]Major product was an alkene. [e]Major product was an alkane. [f]First/second values rerpresent the % yield obtained with/without CuOAc (cat.).

afford a dimetalated intermediate that, in the presence of several chelating neighboring groups, would bring both dimetalated moieties in close proximity, thereby enabling a template-directed oxidative macrocyclization. As depicted in Figure 3a, several metals and oxidants were assessed; however, many of these were unsuccessful because of decomposition of the starting material, protonation of the chloride-derived anion, and/or dimerization. Dilithiation with Li sticks, followed by *trans*-metalation with KO*t*-Bu presumably generated an organodipotassium intermediate that was subsequently oxidized with diphenyl diselenide to afford **8** (91%) containing the complete macrocyclic backbone of **2**. We believe that this template-directed macrocyclization, which presumably occurs via polar or SET mechanisms in accord with Sarpong's findings,[7b] is a new example of an oxidative C−N bond formation from a conformationally unrestricted system.

To investigate the scope of this reaction, a small collection of cyclic secondary amines was synthesized as shown in Figure 3b. These conditions afforded 6- to 15-membered cyclic alkoxyamines **9**−**11** and **13** as well as alkoxyhydrazine **12** in very high yields. Interestingly, the anion source could be generated from either a lithium-halogen exchange or a deprotonation of an *o*-tolyl C−H or an aniline N−H. Attempts to prepare a cyclic secondary amine from Boc-protected 12-chlorododecylamine were unsuccessful, suggesting that an in-chain "RCH_2K"-chelating motif is an essential component.

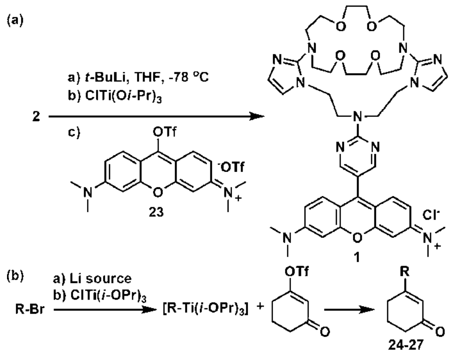
Returning to the synthesis of ionophore **2**, Boc-protected amine **8** was treated with TFA followed by precipitation with aqueous sodium carbonate to quantitatively afford free amine **14**. A solution containing diazonium salt **15** (prepared from the oxidation of 5-bromo-2-aminopyrimidine) was then cannulated into a THF solution of amine **14** with catalytic CuOAc and warmed to reflux temperature to afford **2** (78%). When catalytic CuOAc is not used,[8] the ionophore bromide **2** is obtained in 86% yield. These represent, to our knowledge, the first examples of a secondary amine participating in a Sandmeyer-like or a metal-free aminoarylation from aryldiazonium salts.[9] To investigate the scope of this aminoarylation reaction with and without catalytic copper(I), amines **16**−**22** were prepared in 58−89% yield (Figure 3d). Interestingly, electron-deficient heteroaryl diazonium salts afforded higher % yield than electron-efficient systems.

Having devised an efficient route to ionophore bromide **2** (see Figure 1), our attention turned toward the ionophore-chromophore union as shown in Figure 4a. In prior reports, this union required several steps to afford a K^+ sensor because of the need to construct the chromophore around the ionophore aldehyde carbon.[4] In order to accomplish this union in one transformation, we envisioned an organometallic addition to an activated xanthylium such as the vinylogous sulfonate **23**. Indeed, treatment of **2** with *t*-BuLi, followed by ClTi(O*i*-Pr)$_3$, delivered the corresponding organotitanium reagent. Quenching this anion with a chilled solution of xanthylium triflate **23** (prepared from the corresponding xanthone)[10] gave **1** in 82% yield. Since there are no examples, to our knowledge, of organotitanium additions to vinylogous systems in the literature, the general utility of this transformation was further demonstrated as shown in

(8) CuOAc was chosen over CuCN, as the undesired benzonitrile was observed as a minor product and poisonous HCN is a likely byproduct. When stoichiometric CuCN was used, the benzonitrile was obtained in 60% yield. Beletskaya's conditions afforded the benzonitrile in 89% yield: Beletskaya, I. P.; Sigeev, A. S.; Peregudov, A. S.; Petrovskii, P. V. *J. Organomet. Chem.* **2004**, *689*, 3810–3812.

(9) The Sandmeyer reaction implies a radical process involving Cu(I) or other metals. Presumably, this happens with this Cu(I) version. However, metal-free aminoarylations occur with aryldiazonium salts using thiols, water, or iodide. It is not known, to our knowledge, whether these metal-free versions occur via a radical, *ipso* substitution, or dissociative mechanism. For metal-free versions, see: Filimonov, V. D.; Trusova, M.; Postnikov, P.; Krasnokutskaya, E. A.; Lee, Y. M.; Hwang, H. Y.; Kim, H.; Chi, K.-W. *Org. Lett.* **2008**, *10*, 3961–3964.

(10) Kenmoku, S.; Urano, Y.; Kojima, H.; Nagano, T. *J. Am. Chem. Soc.* **2007**, *129*, 7313–7318.



R-Br	Li source	compd	R	% yield
Ph-Br	*t*-BuLi	24	Ph	84
4-pyridyl-Br	*t*-BuLi	25	4-pyridyl	89
c-C_6H_{11}-Br	Li sticks	26	*c*-C_6H_{11}	78
s-Bu-Br	Li sticks	27	*s*-Bu	74

Figure 4. (a) One-step synthesis of sensor **1** from ionophore bromide **2** via an organotitanium intermediate to vinylogous sulfonate **23**. (b) Cyclohexenones **24**−**27** were prepared with an average yield of 81% with complete chemo- and regioselectivity, thereby demonstrating the utility of this organotitanium addition to a model vinylogous sulfonate.

Figure 4b. Organotitanium reagents (1.0 equiv) derived from aryl or alkyl bromides were added to a model vinylgous sulfonate and afforded 3-substituted cyclohexenones **24**−**27** in an average yield of 81% with expected control of chemo- and regioselectivity.

The K^+ sensitivity and specificity of sensor **1** were compared to reference sensor TAC-Red[4c] as shown in Figure 5. TAC-Red and **1** (each at 7 μM) were dissolved in pH 7 HEPES buffer balanced with KCl/NaCl to maintain constant ionic strength at 200 mM. The structurally simplified **1** was slightly more sensitive to K^+ than TAC-Red as shown in Figure 5a. Figure 5b shows that **1** was remarkably more selective for K^+ versus Cs^+ or Rb^+ than TAC-Red and had comparable low sensitivity to Na^+, Li^+, Mg^{2+}, and Ca^{2+} (the latter three ions not shown in Figure 5b). Finally, **1** had comparable pH insensitivity to TAC-Red at pH greater than 6 as shown in Figure 5c. These results are likely attributed to **1** having a smaller ionophore cage that excludes the larger cations like Cs^+ and Rb^+ yet brings Lewis basic nitrogen and oxygen atoms in a favorable position for K^+ binding.

In summary, the streamlined synthesis reported here, which represents over a 2-fold decrease in steps compared to TAC-Red, affords the functionally superior yet structurally simplified K^+ sensor **1** in 52% overall yield with the longest linear sequence being 4−5 steps. Additionally, this report highlights new ligand and microwave conditions for aminoarylations, a C−N bond-forming oxidative cyclization reaction to generate cyclic secondary alkoxyamines, the first example of secondary amines in Sandmeyer-like reactions, new examples of metal-free aminoarylations, and an efficient route to chromophore conjugates via an organotitanium addition to vinylogous sulfonates. Creating bioconjugates derived

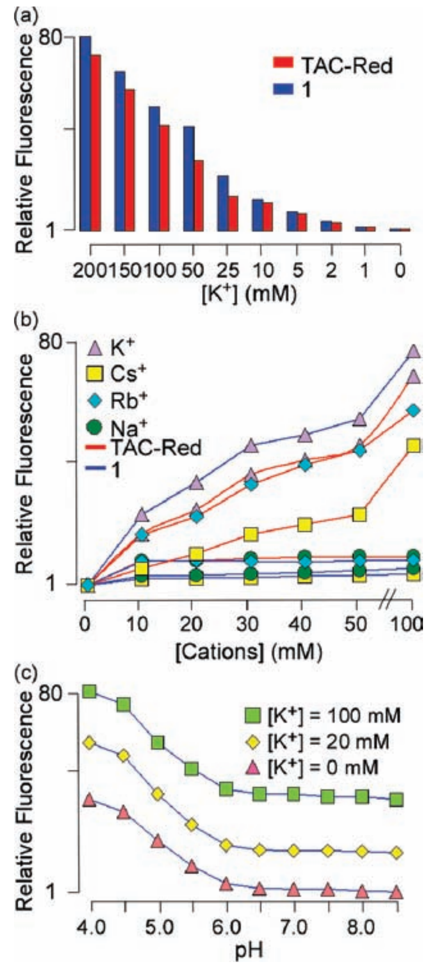


Figure 5. (a) Fluorescence emission at 570 nm of **1** (blue) and TAC-Red[4c] (red) at various [K^+]. (b) Fluorescence at 570 nm of **1** (blue line) and TAC-Red (red line) in the presence of indicated cations. (c) Fluorescence of **1** as a function of pH at indicated [K^+]. Samples in a−c were excited at 480 nm, and solutions were balanced with NaCl or choline chloride to maintain a constant ionic strength of 200 mM.

from sensor **1** to measure [K^+] in major molecular, cellular, translational, and clinical applications will be reported in due course.

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Supporting Information Available: Experimental procedures and analytical data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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