

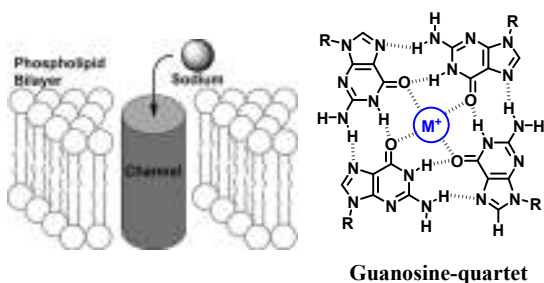
# A G-Quadruplex that Functions as a Transmembrane Na<sup>+</sup> Transporter

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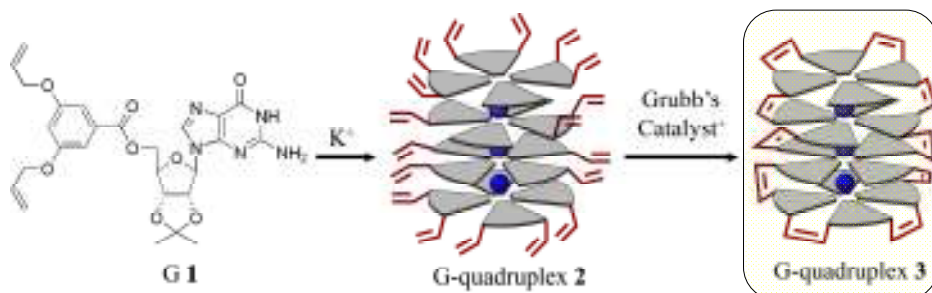
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## I. Introduction

The goal of the present research is to design and build synthetic ion channels. The design of synthetic channels and transmembrane transporters may provide new ion sensors, catalysts and anti-microbial agents.<sup>1</sup> Our approach has been to use lipophilic guanosine to build synthetic ion channels.<sup>2,3</sup>



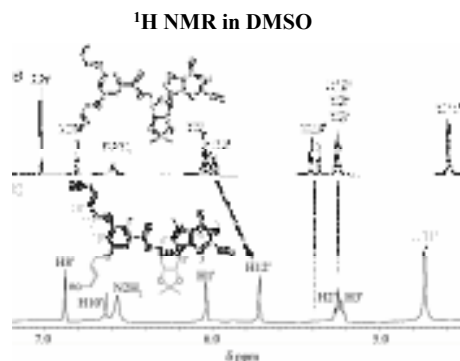
## II. Design and Strategy



## III. Metathesis and Characterization

G-quadruplex formation of G 1 was characterized by <sup>1</sup>H NMR and CD spectroscopy. Olefin metathesis was carried out on the preformed G-quadruplex 2 in CH<sub>2</sub>Cl<sub>2</sub>. Monitoring the reaction by NMR showed the disappearance of the terminal olefinic protons and the appearance of new signals for the secondary olefins within the metathesis product 3.

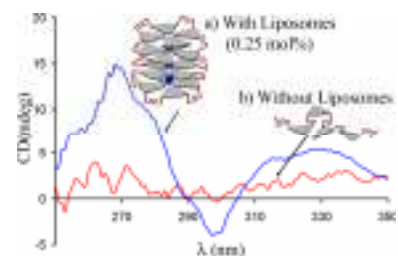
ESI-MS shows a parent peak for a triply charged (G 3)<sub>16</sub>•3K<sup>+</sup> as the main peak (m/z=2766.3). Pulsed-field gradient NMR experiments in the competitive solvent DMSO-d<sub>6</sub> confirmed that metathesis product [G]<sub>16</sub> 3 was the size of a hexadecamer.<sup>4</sup>



## IV. Structure in Phospholipid Membranes

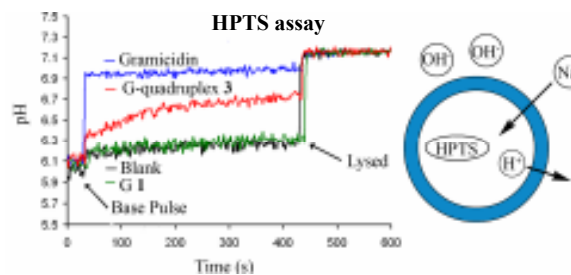
Metathesis product 3 gave the diagnostic CD spectrum for a G-quadruplex when added to an aqueous solution of EYPC liposomes, indicating that [G]<sub>16</sub> 3 can fold into this secondary structure within the phospholipid bilayer.

## CD Spectra in 10 mM Na phosphate buffer



## V. Na<sup>+</sup> Transport Activity

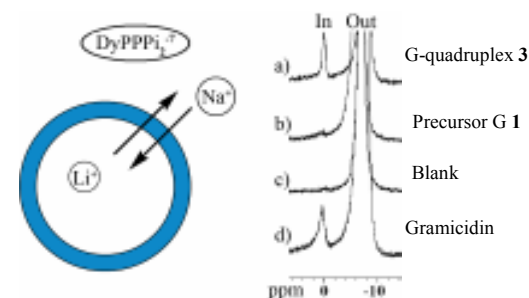
G-quadruplex 3 was first studied using a base-pulse assay to show ion transport. Liposomes were prepared with a pH sensitive dye (HPTS) on the inside. Addition of an exogenous base led to a rapid increase in the internal pH of these liposomes when they were in the presence of metathesis product 3 and positive control (gramicidin). Gramicidin is a protein that functions as a Na<sup>+</sup> channel.



## IX. References

1. For detailed description of experiments see: Kaucher, M. S. Harrell Jr., W. H. and Davis, J. T. *J. Am. Chem. Soc.* **2005**, accepted.
2. Forman, S. L.; Fetting, J. C.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *J. Am. Chem. Soc.* **2000**, *122*, 4060-4067.
3. Davis, J. T. *Angew. Chem., Int. Ed. Engl.* **2004**, *43*, 668-698.
4. Kaucher, M. S.; Lam, Y. F.; Pieraccini, S.; Gottarelli, G.; Davis, J. T. *Chem. Eur. J.* **2004**, *11*, 164-173.

## VI. <sup>23</sup>Na NMR



<sup>23</sup>Na NMR experiments using an Na<sup>+</sup> NMR shift reagent on the outside of liposomes to distinguish inside and outside liposomes showed direct evidence of Na<sup>+</sup> transport. Liposomes were prepared with Na<sup>+</sup> outside and no Na<sup>+</sup> inside. Metathesized product 3 showed equilibrium of inside and outside Na<sup>+</sup> after 10 minutes indicating Na<sup>+</sup> transport.

## VII. Summary

In conclusion, we have used a strategy that combines non-covalent synthesis and covalent capture to prepare a functional supramolecular assembly, unimolecular G-quadruplex 3, in just two steps from a guanosine derivative. The unimolecular G-quadruplex 3 apparently folds into a conformation that allows it to bind and transport Na<sup>+</sup> ions across phospholipid bilayer membranes.

## VIII. Acknowledgements

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