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Calixarene-mediated liquid membrane transport of choline conjugates 3: The effect of handle variation on neurotransmitter transport

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ABSTRACT

Upper rim phosphonic acid functionalized calix[4]arene affects selective transport of multiple molecular payloads through a liquid membrane. The secret is in the attachment of a receptor-complementary handle to the payload. We find that the trimethylammonium ethylene group present in choline is one of several general handles for the transport of drug and drug-like species. Herein we compare the effect of handle variation against the transport of serotonin and dopamine. We find that several ionizable amine termini handles are sufficient for transport and identify two ideal candidates. Their performance is significantly enhanced in HEPES buffered solutions. This inquiry completes a series of 3 studies aimed at optimization of this strategy. In completion a new approach towards synthetic receptor mediated selective small molecule transport has emerged; future work in vesicular and cellular systems will follow.

Introduction

While new lead discovery is a chief pursuit towards the development of new therapeutics, we opine that drug delivery is a critically important complement. For example, what can be done with the discovery of a high-impact (in vitro) lead target that the cell membrane (in vivo) resists? Chemical modification is one route and formulation another. But for each subsequent stage of the pipeline, from discovery to clinical approval the path narrows. Are there parallel avenues in any stage that would allow more leads to progress down the pipeline? Drug transport doesn't have to binary operator, particular toxic compounds that have significant efficacy, could be dosed at a lower level if enhanced transport methods can be found. We see many ingenious systems including those that received clinical approval, but challenges that have been indentified[1,2] continue to receive attention,[3] especially at the blood-brain barrier.[4]

The use of calixarene and resorcinarene cavitands as selective shuttles is a novel approach when compared to covalent attachment of a drug to a delivery vehicle or emulsification in lipid micelles, for examples. We aim to develop: 1) a receptor that localizes in the cell membrane, 2) a receptor that doesn't harm the cell nor cause non-specific leakage, 3) a complementary receptor-handle system that can selectively transport a variety of handle-payload conjugates (enhancing the transport of many classes of drugs) and 4) a receptor that transports far more than one equivalent of payload. Valinomycin and nonactin provide the biological inspiration for what is possible (all of our aims).[5,6] These aims, while ambitious could not only rejuvenate interesting candidates, but perhaps could one day result in decreasing dosing if indeed a general method to enhance transport is found. Recent work expands drastically the relationship between supramolecular chemistry and bio-inspired functionality[7] and new applications continue to advance.

Our contribution follows from a rich history of supramolecular host-guest chemistry and concepts, primarily related to resorcinarene cavitand host binding[8] and catalysis.[9] We have discovered that these systems function in aqueous lipid systems as a host,[10] as a platform for switching,[11] and we know too that these molecules distribute themselves evenly in lipid bilayer systems.[12] Our colleagues demonstrated remarkable function as surface bound receptors for protein sensing[13] and most interestingly as facilitating endocytosis of guest molecules![14] These properties have been developed under constraints of the host, as they are largely incompatible with pure water environments. We reported that resorcinarene cavitands were limited in their ability to transport due to their strong binding of complementary handles such as choline.[15] As it happens they were excellent extractors but terrible transporters. Calixarene species with ionizable groups on the other hand ultimately transported several payloads, while proving to be moderate extractors and weak binders. This work built on a variety of prior reports with trimethylammonium guests.[16–19] In this context several important papers using bulk liquid membranes have

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resulted in the development of synthetic receptors that transport metal ions,[20,21] can carry out enantioselective transport of amino acids[22] and principles of dynamic combinatorial chemistry have uncovered new transporters.[23]

As reported phosphonic acid **1**[24,25] could effect transport across a bulk liquid membrane without a pH gradient, whereas lower rim functionalized carboxylates calix[4]arene **2**[26] and calix[6]arene **3**[27] responded favorably to an inverse acid gradient (Fig. 1). We hypothesize that calixarenes provide ion-ion interactions with choline that overcome two-phase extraction, but are lenient enough to release a payload into a welcoming receiving phase. Whereas resorcinarene cavitands were unable to accomplish this essential latter requirement. While we have suspended our transport efforts with carboxylates, we have confidence that they can serve as a backup should phosphonic acid **1** present problems in more relevant cell based assays. While pursuing this, we found complementary use for the calix[6]arene hexacarboxylic acid towards binding of Pb, Sr and Ba with a new calix[6]arene octahedral geometry occurring.[28–30]

Recently we reported that calixarenes are promising receptors for liquid membrane transport of choline-fluorophore conjugates.[15] Our results indicated that the presence of ionizable, preorganized functional groups such as those on a calixarene scaffold provide effective transport of choline-fluorophore conjugates. Lower rim carboxylic acid **2**, **3** and upper rim phosphonic acid **1** groups were sufficient to transport payloads appended with a trimethylammonium handle such as that found in choline (O-ethylene trimethylammonium). Tetraphosphonic acid calix[4]arene **1**[24,25] was capable of transporting choline conjugates *without* a complementary, inverse pH gradient and became our sole focus.[15] Our second study examined the nature of the payload and we found many drug and drug like entities were efficiently transported through a liquid membrane – usually at rates far superior compared to controls lacking a calixarene transporter.[31] Some limits obviously emerged, but also exciting results showing that serotonin and dopamine with a smaller ammonium handle were also transported. Concurrently, we serendipitously uncovered a third useful handle in the form of an ammonium dicarboxylate. With three potential handles at our disposal we wanted to complete our initial work to directly compare the effect of handle on transport efficiency. Serotonin and dopamine were chosen to compare endogenous ammonium, and chemically introduced trimethylammonium and ammonium dicarboxylate handles side by side. Simultaneously we explored the role of HEPES buffer on these events as we previously noted a surprising advantage while using it for one payload.[31]

These results we believe complete our optimization of handle and host and afford us some variety of introducing a handle onto a new membrane resistant drug-candidate. With the versatility of 3 hosts (**1**–**3**) and 3 functional handles (see below) we have a small tool set at our disposal as we look for answers to the drug transport problem.

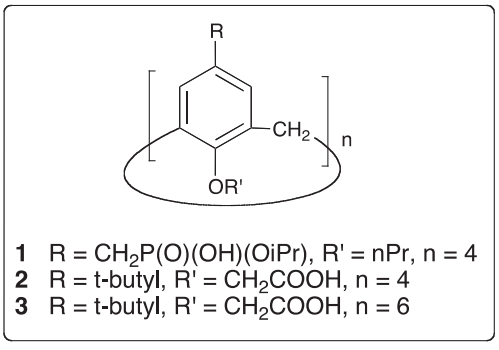

Fig. 1. Structure of upper phophonic acid calix[4]arenen **1** and lower carboxylic acid calix[4]arene **2** and calix[6]arene **3**.

Discussion

Trimethylammonium dopamine **6** was produced in fair yields upon exhaustive methylation of dopamine hydrochloride (Scheme 1, for full details see ESI). Under our reaction conditions no phenolic ethers were detected, our recrystallization procedure easily removes excess potassium carbonate, but does not afford high yields at this time. Crude NMR during the reaction indicates clean conversion, but some product is likely lost when separating the solid trimethylammoniums from carbonate. An ammonium dicarboxylate handle was installed in two straightforward steps starting with addition of two *t*-butyl acetates to the free amine of **5**. Subsequent removal of *t*-butyl groups using TFA afforded **7** as a TFA salt.

Following the same two protocols for preparation of dopamine derivatives **6** and **7**, analogs of serotonin were prepared (Scheme 2). Exhaustive methylation of serotonin hydrochloride **8** with methyl iodide gave readily isolated trimethylammonium serotonin **9** in fair, but unoptimized yield. Ammonium dicarboxylate **10** was prepared in two steps starting with addition of two *t*-butyl acetates to the free amine of **8**. Subsequent removal of *t*-butyl groups using TFA afforded **10** as a TFA salt. We will refer to these compounds as ammonium dicarboxylates from this point forward, when dissolved in water - this is a more accurate representation of their likely protonation state.

With this matrix of 2 neurotransmitters with three handle, we screened them against tetraphosphonic acid calix[4]arene receptor **1** using a 3-phase U-tube apparatus. Screens were conducted in both water and 10 mM HEPES Buffer (pH 7.4). A detailed description of the apparatus as well as representative calibration curves are found in the ESI, we graph and discuss the results herein.

As we reported, dopamine **5** had a transport flux of $1.18 \times 10^{-4} \pm 0.02 \times 10^{-4}$ μmoles cm^{-2} min^{-1} in water with virtually no transport in the absence of host (Fig. 2 and Table 1).[31] Transport was enhanced 4.6 times when HEPES buffered source and receiving phases were used. The enhanced transport in HEPES was a surprise to us. The exact mechanism of enhancement is unclear at this time, but one small effect might be on the protonation/deprotonation of the host at the interfaces due to buffering. Increased salt concentration also could play a role. We then examined the effect of changing the charged ammonium handle of **5** to the larger trimethylammonium handle of **6**. This handle was the basis for our first two reports on this subject. A trimethylammonium handle had considerable reach in its ability to transport a variety of fluorophores, drug-like and drug molecules when combined with hosts **1**–**3**.

Comparing **5** vs. **6** in water we note that transport is 1.6 times more efficient for **6**. When switching to HEPES, **6** is transported 1.7 times more than **5.** We then conducted the same experiments with an ammonium dicarboxylate handle **7**. Comparing **5** vs. **7** we see a drastic decrease in transport (0.17 water, 0.15 Hepes). In the cases of **5** and **6** a comparison of the control experiment with no host to an experiment with host present is virtually meaningless, in water and HEPES both guests **5** and **6** had little or no detectable transport after 72 h under control conditions; the presence of host **1** was the required ingredient for transport. For guest **7** however, the change in the nature of the substrate resulted in non-zero control transport, in these cases while host mediated transport is much lower than for **5** and **6**, we find that host **1** enhances guest **7** transport (2.5 times in water, 5.1 times in HEPES).

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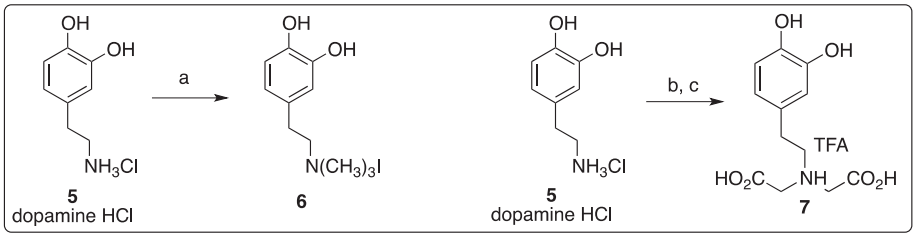
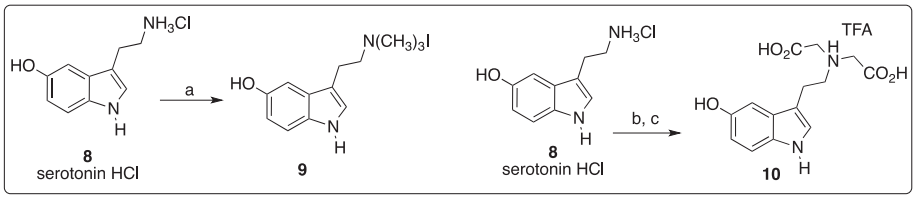


Scheme 1. Structures and syntheses of trimethylammonium dopamine **6** and dicarboxylate ammonium dopamine **7**. Reaction conditions and yields a) CH_3I, K_2CO_3, EtOAc, 67%; b) $BrCH_2CO_2tBu$, K_2CO_3, acetonitrile, 52%; c) TFA, DCM, 81%.



Scheme 2. Structures and syntheses of trimethylammonium serotonin **9** and dicarboxylate ammonium serotonin **10**. Reaction conditions and yields a) CH_3I, K_2CO_3, EtOAc, 54%; b) $BrCH_2CO_2tBu$, K_2CO_3, acetonitrile, 61%; c) TFA, DCM, 40%.

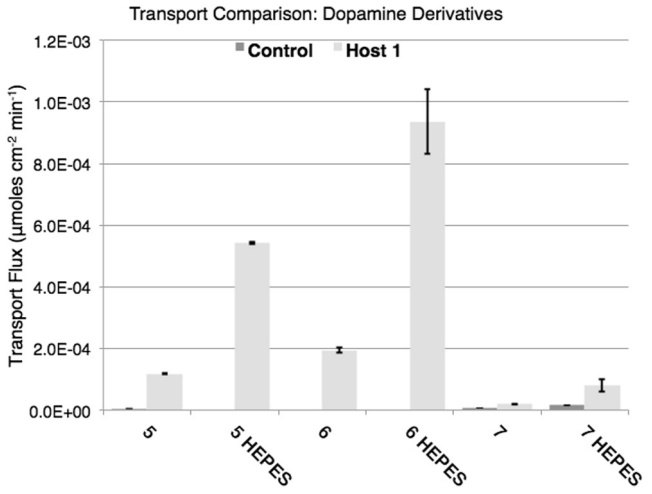


Fig. 2. Transport comparison of dopamine **5**, trimethylammonium dopamine **6** and dopamine ammonium dicarboxylate **7** in water and 10 mM HEPES (pH 7.4) using U-tube transport apparatus (See ESI for full details), briefly: organic phase 10 mL (0.5 mM **1**) in DCM or DCM control, source phase 4 mL aqueous solution of substrate (5.0 mM), after stirring organic phase at 400 rpm for 72 h aliquots were removed and analyzed against UV–vis calibration curve of substrate, transport flux is reported (μmoles cm^{-2} min^{-1}) as an average of duplicate experiments and error bars are shown as the maximum deviation from the mean. Controls, when non-zero are shaded darker.

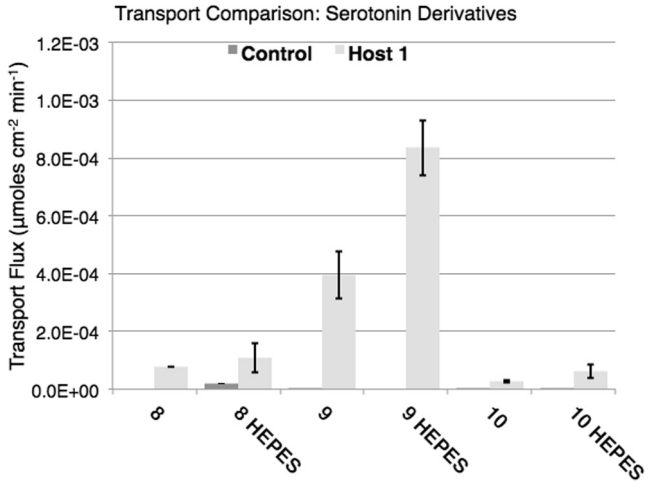


Fig. 3. Transport comparison of serotonin **8**, trimethylammonium serotonin **9** and serotonin ammonium dicarboxylate **10** in water and 10 mM HEPES (pH 7.4) using U-tube transport apparatus (See ESI for full details), briefly: organic phase 10 mL (0.5 mM **1**) in DCM or DCM control, source phase 4 mL aqueous solution of substrate (5.0 mM), after stirring organic phase at 400 rpm for 72 h aliquots were removed and analyzed against UV–vis calibration curve of substrate, transport flux is reported (μmoles cm^{-2} min^{-1}) as an average of duplicate experiments and error bars are shown as the maximum deviation from the mean. Controls, when non-zero are shaded darker.

We then conducted an analogous study with serotonin and derivatives **8**–**10** (Fig. 3). Previously reporting that host **1** transported serotonin **8** with a flux of $7.73 \times 10^{-5} \pm 0.10 \times 10^{-5}$ - μmoles cm^{-2} min^{-1}, enhancement to transport was observed in HEPES, however the difference falls within the error of the measurement. Directly comparing the trimentylammonium **9** to **8** in water, as was the case with dopamine (**6** vs **5**, Fig. 3) we see a drastic advantage, 5.5 times more transport is observed. When making

Table 1
Transport flux (μmoles cm^{-2} min^{-1}) reported for **5**–**7** as an average of duplicate experiments and maximum deviation from the mean (graphed in Fig. 2).

Guest/Conditions	Control	Max dev.	Host 1	Max dev.
5 water	5.34×10^{-6}	0	1.18×10^{-4}	2.00×10^{-6}
5 HEPES	0	0	5.42×10^{-4}	4.10×10^{-6}
6 water	0	0	1.94×10^{-4}	8.60×10^{-6}
6 HEPES	0	0	9.35×10^{-4}	1.06×10^{-4}
7 water	7.70×10^{-6}	8.2×10^{-7}	1.96×10^{-5}	1.23×10^{-6}
7 HEPES	1.56×10^{-5}	8.2×10^{-7}	8.04×10^{-5}	2.13×10^{-5}

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Table 2
Transport flux (μmoles cm^{-2} min^{-1}) reported for **8**–**10** as an average of duplicate experiments and maximum deviation from the mean (graphed in Fig. 3).

Guest/Conditions	Control	Max dev.	Host 1	Max dev.
8 water	0	0	7.73×10^{-5}	1.00×10^{-6}
8 HEPES	1.87×10^{-5}	2.28×10^{-7}	1.09×10^{-4}	5.13×10^{-5}
9 water	4.65×10^{-8}	0	3.94×10^{-4}	8.12×10^{-5}
9 HEPES	0	0	8.35×10^{-4}	9.58×10^{-5}
10 water	1.37×10^{-7}	0	2.83×10^{-5}	4.92×10^{-6}
10 HEPES	2.73×10^{-7}	4.10×10^{-7}	6.27×10^{-5}	2.38×10^{-5}

the comparison in HEPES a 7.7 times enhancement is observed. The advantage for the trimethylammonium handle again is obvious for pairing with host **1**. Ammonium dicarboxylate **10** shows the least efficient transport, comparing to **8** we find a decrease in transport 0.4 (water), 0.6 (HEPES). In almost all cases negligible transport of serotonin and derivatives **8**–**10** was observed in the absence of host **1**. Selective transport is demonstrated in each example (Table 2).

Conclusion

We clearly identify the ethylene trimethylammonium group as having a distinct advantageous for selective payload transport in the presence of calix[4]arene phosphonic acid **1**. Similarly the smaller (and native to dopamine and serotonin) ethylene ammonium handle responds favorably to calixarene mediated transport. In all cases host provides a distinct enhancement in transport compared to control experiments that lack host. A third handle: ammonium dicarboxylate works to a smaller degree. In all cases, HEPES buffered aqueous phases that better mimic biological environs respond favorably compared to water. In no cases was an inverse ion gradient required to carry out selective transport. The phosphonic acid group likely is in just the right pK_A range over the timeframe of study. Previous work with carboxylic acids differed and in that case an acidified receiving phase was necessary for significant transport. For our future work these three handles are viewed as tools to attach to membrane resistant drug candidates to serve as a host-guest transport pair. Tuning of calixarenes to lodge in the cell membrane would then provide a platform for multiple transport events, selective for molecules with these complementary handles. Installation of an ethylene trimethylammonium group is usually accomplished through ether or amine linkages, or through direct methylation of a terminal amine. These synthetic routes are straightforward and in complex molecules of interest, the potential to install them away from a pharmacophore provides a means for using this system readily. The combination of these groups broadens our options as we approach more pressing targets. In our next study, we wish to enhance drug transport by guest modification, followed by cell-based screening. While we can hope that these 3 handles will not interfere with a drug's mode of action or efficacy at a molecular target - this certainly can't be assured until tested. When we get to that bridge a careful examination of how a drug binds to its target along with feasible modifications will have to be identified, then the system tested. With three handles that all show enhanced transport - our outlook improves. We can approach these problems better with these newly reported options.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.05.009.

References

1. Langer R. *Science*. 1990;249:1527.
2. Pardridge WM. *Drug Discovery Today*. 2007;12:54.
3. Hersh DS, Wadajkar AS, Roberts N, et al. *Curr Pharm Des*. 2016;22:1177.
4. Weiser JR, Saltzman WM. *J Controlled Release*. 2014;190:664.
5. Neupert-Laves K, Dobler M. *Helv Chim Acta*. 1975;58:432.
6. Späth A, König B. *Beilstein J Org Chem*. 2010;6:32.
7. Tu Y, Peng F, Adawy A, Men Y, Abdelmohsen LKEA, Wilson DA. *Chem Rev*. 2016;116:2023.
8. Biros SM, Rebek JJ. *Chem Soc Rev*. 2007;36:93.
9. Iwasawa T, Hooley RJ, Rebek J. *Science*. 2007;317:493.
10. Schramm MP, Hooley RJ, Rebek J. *J Am Chem Soc*. 2007;129:9773.
11. Kim YJ, Lek MT, Schramm MP. *Chem Commun (Camb)*. 2011;47:9636.
12. Feher KM, Hoang H, Schramm MP. *New J Chem*. 2012;36:874.
13. Liu Y, Liao P, Cheng Q, Hooley RJ. *J Am Chem Soc*. 2010;132:10383.
14. Ghang Y-J, Schramm MP, Zhang F, et al. *J Am Chem Soc*. 2013;135:7090.
15. Adhikari BB, Fujii A, Schramm MP. *Eur J Org Chem*. 2014;2014:2972.
16. Shinkai S, Araki K, Manabe O. *J Am Chem Soc*. 1988;110:7214.
17. Ogoshi T, Kanai S, Fujinami S, Yamagishi T-A, Nakamoto Y. *J Am Chem Soc*. 2008;130:5022.
18. Daze KD, Pinter T, Beshara CS, et al. *Chemical Science*. 2012;3:2695.
19. Tao H, Cao D, Liu L, Kou Y, Wang L, Meier H. *Sci China Chem*. 2012;55:223.
20. Nakatsuji Y, Okahara M. *Pure Appl Chem*. 1993;65:557.
21. Wright AJ, Matthews SE, Fischer WB, Beer PD. *Chem Eur J*. 2001;7:3474.
22. Breccia P, Van Gool M, Perez-Fernandez R, et al. *J Am Chem Soc*. 2003;125:8270.
23. Perez-Fernandez R, Pittelkow M, Belenguer AM, Lane LA, Robinson CV, Sanders JKM. *Chem Commun*. 2009;3708.
24. Ohto K, Yamasaki T, Inoue K. *Ars Separatoria Acta*. 2006;4:96.
25. Ohto K, Ota H, Inoue K. *Solvent Extr Res Dev Japan*. 1997;4:167.
26. Ohto K, Tanaka H, Ishibashi H, Inoue K. *Solvent Extr Ion Exch*. 1999;17:1309.
27. Oshima T, Suetsugu A, Baba Y, Shikaze Y, Ohto K, Inoue K. *J Membr Sci*. 2008;307:284.
28. Adhikari BB, Ohto K, Schramm MP. *Chem Commun*. 2014;50:1903.
29. Adhikari BB, Zhao X, Derakhshan S, Schramm MP. *Dalton Trans*. 2014;43:15560.
30. Adhikari BB, To C-A, Iwasawa T, Schramm MP. *Supramol Chem*. 2015;27:724.
31. Adhikari BB, Roshandel S, Fujii A, Schramm MP. *Eur J Org Chem*. 2015;2015:2683.