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Calixarene-Mediated Liquid Membrane Transport of Choline Conjugates 2: Transport of Drug-Choline Conjugates and Neurotransmitters

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Abstract

Lower rim carboxylic acid calix[*n*]arenes and upper rim phosphonic acid functionalized calix[4]arenes effect selective transport of distinct molecular payloads through a liquid membrane. The secret to this success lies in the attachment of a receptor-complementary handle. We find that the trimethylammonium ethylene group present in choline is a general handle for the transport of drug and drug-like species. Furthermore, neurotransmitters possessing ionizable amine termini are also transported. Some limitations to this strategy have been uncovered as payloads become increasingly lipophilic. These developments reveal new approaches to synthetic receptor-mediated selective small molecule transport in vesicular and cellular systems.

Keywords

Medicinal chemistry; Drug design; Drug delivery; Receptors; Calixarenes; Dopamine; Serotonin

Introduction

We recently reported that calixarenes are promising receptors for liquid membrane transport of choline-fluorophore conjugates.^[1] Our results indicated that the presence of ionizable, pre-organized functional groups such as those on a calixarene scaffold enable effective transport of choline-fluorophore conjugates. Moreover, the tetrakisphosphonic acid calix[4]arene **1** was capable of transporting choline conjugates without a complementary inverse pH gradient.

Our ultimate interest in this work stems from a desire to overcome drug transport challenges inherent to humans. Indeed, drug delivery is an important complement to drug discovery, especially for high-impact (in vitro) lead targets that (in vivo) the cell membrane resists. Many ingenious systems have been developed and some have received clinical approval, but challenges remain.^[2] The use of calixarene and resorcinarene cavitands is a novel approach relative to covalent drug attachment to delivery vehicles or emulsifications in lipid micelles.

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We aim to develop: 1) a receptor that localizes in the cell membrane, 2) a receptor that does not harm the cell or cause non-specific leakage, 3) a complementary receptor handle system that can selectively transport a variety of handle-payload conjugates and 4) a receptor that transports more than one equivalent of payload. Valinomycin and nonactin provide the biological inspiration for what is possible.^[3,4]

Our contribution stems from a rich history of supramolecular host–guest chemistry and concepts, primarily related to resorcinarene cavitand host binding^[5] and catalysis.^[6] We have discovered that these systems function in aqueous lipid systems as hosts,^[7] and as platforms for switching,^[8] and we know too that these molecules distribute themselves evenly in lipid bilayer systems.^[9] Our colleagues have demonstrated remarkable functions for these systems as surface bound receptors for protein sensing^[10] and, most interestingly, as facilitators of endocytosis for guest molecules!^[11] These properties have been developed under constraints of the host, as they are largely incompatible with pure water environments. We had hoped that resorcinarene cavitands would be a suitable prototype for transport. However, limitations stemming from their strong binding of complementary handles such as choline indicated that efficient guest release would be difficult to achieve.^[1] Consequently, calixarene species with ionizable groups were identified as being capable of extraction and, ultimately, transport. This work built on a variety of earlier reports with trimethylammonium guests.^[12] In this context, several important papers using bulk liquid membranes have resulted in the development of synthetic receptors that transport metal ions,^[13] and can carry out enantioselective transport of amino acids.^[14] Clearly then, the principles of dynamic combinatorial chemistry have uncovered new transporters.^[15] As mentioned, phosphonic acid **1** was found to effect transport across a bulk liquid membrane without a pH gradient, whereas lower-rim functionalized carboxylates calix[4]arene **2** and calix[6]arene **4** responded favorably to inverse acid gradients. 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 are unable to accomplish this essential latter requirement.

These initial results encouraged us to further explore the range of possible payloads suitable for delivery. Herein our work with drug and drug-like molecules is reported.

Results and Discussion

We employed upper-rim tetrakisphosphonic acid calix[4]-arene **1**^[16] and a homologous series of lower rim carboxylic acid calix[4] **2**,^[17] calix[5] **3**^[18] and calix[6] **4** – arenes (Figure 1). BOC protected tryptophan–choline **5**, acetaminophen–choline **6**, ibuprofen–choline **7**, tamoxifen–choline **8**, dimethylresveratrol–choline **9** and bentiromide–choline **10** were selected as guest–choline conjugates. This collection of molecules afforded us easy access to a variety of functional groups, lipophilicities and molecular complexities enabling an initial study of the limits of selective drug-conjugate transport. These are some of the central issues to be considered in solving drug-transport problems.

Two Phase Solvent Extraction of Tryptophan-Choline 5

Extraction, the logical preamble to transport was studied first. Accordingly, we initially explored receptor-mediated extraction of tryptophan–choline **5** from an aqueous solution (Table 1). We exposed a stirring solution of receptor (0.5 mM, DCM, 160 rpm) to an equal volume of substrate in water and stirred for 24 h. An aliquot of the aqueous phase was then removed and absorbance measured to determine the net decrease in substrate concentration. We found that host **1** was ineffective at extracting guests below 0.5 mM, but showed modest extraction efficiencies at elevated guest concentrations. At low guest concentrations the competitive partitioning of host into the guest aqueous layer appears to be a significant factor.

Carboxylic acid receptors **2** and **4** were more efficient hosts for extracting guest **5** into the organic layer. Appreciable extents of extraction were measured at all guest concentrations and significant extraction efficiencies were observed at high guest concentrations. For **4** at a ratio of 6:1 guest to host, the extraction efficiency was found to be 100%; under these conditions each host is occupied by a guest.

Three-Phase Transport of Tryptophan-Choline 5

We then explored the three-phase transport of **5** facilitated by four different receptors under different substrate concentrations (low and high) and different receiving phase conditions (neutral and acidic) (see Supporting Information for representative UV data and full details). Under neutral conditions we found that phosphonic acid **1** efficiently enhanced transport flux of **5** on the order of 4.5-fold faster than in the control when guest concentration was low (Table 2). The remaining receptors were all less efficient transporters than the control, a finding that is consistent with our extraction experiments (Table 1). Indeed hosts **2–4** proved to be excellent extractors, but failed to deliver the guest into the receiving phase under neutral conditions.

Notably, transport characteristics changed drastically when the receiving phase was acidified. Host **1** remained an efficient transporter vs. control, but calix[4]arene **2** and calix[5]arene **3** carboxylic acids became exceptional transporters, each facilitating the delivery of over 9 times more guest vs. control at a guest concentration of 0.5 mM. Calix[6]arene hexacarboxylic acid **4** was also efficient at guest transport when the receiving phase was acidified. Perhaps the pre-organization of **2** and **3** instills some transportational advantages that **4** simply cannot offer due to its dynamic conformation in solution. Indeed, it is not simply the number of functional groups that matters to the transport process; calix[5]arene may offer the best ratio of fixed conformation and number of functional groups.

Transport of Drug Conjugates

Receptors **1** and **2** were selected for further studies to understand the transport of similar drug–choline conjugates acetaminophen–choline **6** and ibuprofen–choline **7**. We found that both receptors can facilitate transport of both guests, but differential behaviors emerge resulting from structural differences between guests (Table 3). Guest **6** is most effectively transported by calix[4]arene carboxylic acid **2** at low pH, whereas the slightly more

lipophilic **7** is transported best by calix[4]arene phosphonic acid **1** at low pH. Although these results are empirical, a complementary set of hosts continues to demonstrate advantages. Host **1** continues to show broad guest compatibility in neutral environments routinely giving enhanced transport. In this experiment **1** clearly demonstrates some advantages over carboxylic acid host **2** for transporting lipophilic guests. Hosts such as **2**, may serve as more potent extractors of lipophilic guests (see below). Additionally, host **2** shows remarkable transport enhancements when the receiving phase is acidified for a variety of guests.

Extraction and Transport of Lipophilic and More Complex Substrates

Amphiphilic substrates **5–7** were found to preferentially reside in the aqueous phase in both extraction and transport experiments. However, in the presence of ionizable calixarenes **5–7** could be enticed into *and* through a hydrophobic environment. Next, we studied a more lipophilic choline-drug conjugate, tamoxifen-choline **8**. Conjugate **8** was synthesized by single *N*-methylation of tamoxifen. We first evaluated the binding of **8** with **1** and **4** in CDCl₃ by ¹H NMR (See Supporting Information for complete details). NMR changes indicated the formation of fast exchange complexes between **8** and both **1** and **4** in CDCl₃. The signal attributed to the trimethylammonium moiety was broadened and shifted upfield. We attempted U-tube experiments using a saturated solution of **8** (63 μM)^[19] in milliQ water and found no evidence of transport using either 0.5 mM of **1** (neutral or acidic receiving phase) or **2** (neutral receiving phase). Instead, we observed significant partitioning, as one would expect for a hydrophobic guest – indeed 46–49 % of **8** was extracted into DCM in the absence of host. Host **1** enabled an additional extraction of 30.14 ± 1.66% under neutral conditions and 14.86 ± 1.54% under acidic receiving phase conditions. Compound **2** enabled an additional 14.39 ± 0.46% extraction. We then turned our attention to the parent compound, tamoxifen, which lacks one *N*-methyl group. We prepared the tamoxifen HCl salt in the hopes of slightly enhancing its solubility relative to trimethylammonium analogue **8**. Unfortunately, the HCl salt also displayed limited water solubility (less than 0.1 mM). We observed identical behavior – no transport and significant partitioning into DCM in the absence of host, contrasted with enhanced extraction into DCM in the presence of host **1**.

Next, we examined resveratrol analogue **9**,^[20] (containing two aromatic rings) which has enhanced water solubility (0.5 mM). This substrate showed decreased transport vs. control under a variety of conditions indicating extraction into the organic phase. However an acidic receiving phase and combination of phosphonic acid host **1** or carboxylic acid host **2** enhanced the transport of this lipophilic substrate by 3.4- and 2.6-fold, respectively, vs. control (Table 4).

Finally, we turned our attention to a large polar payload, bentiromide–choline **10**. We were encouraged to see transport enhancement in the presence of receptors **1** and **2** under neutral and acidic conditions. We have previously demonstrated the significance and selectivity of the choline handle.^[1] However, given the number of ionizable groups on **10**, we decided to confirm that the choline handle is required for transport for this polyfunctional molecule. Parent compound bentiromide^[21] presented some challenges associated with its poor solubility. Indeed, precipitation of bentiromide at the solvent/environment interface was noted. The background transport rate was higher for bentiromide compared to our modified

10. This is likely due to enhanced solubility in the organic phase relative to **10**, which is characterized by a trimethylammonium group. Very slight enhancement in transport was noted in the presence of **1**. In these measurements we used the receiving phase to calculate flux, but we also measured the decrease of guest concentration in the source phase. Comparisons of these numbers allowed us to differentiate transport vs. extraction. In the case of bentiromide, the source phase lost 50% of its initial concentration due to precipitation and partitioning; the receiving phase accounted for about 7% of this 50% loss. Comparisons of transport and extraction between bentiromide and **10** are complicated by this fact. Nevertheless, our results with **10** nicely illustrate the potential of this host-conjugate + guest transport system. Clearly, enhanced transport of a compound characterized by both significant molecular mass and a variety of drug-like features is achievable. Additionally, the trimethylammonium group of **10** was found to result in enhanced water solubility as well as differential behaviors in the presence or absence of host. Despite the imperfect comparisons between **10** and bentiromide, we think this latter result presents opportunities for other drug candidates that partition into lipophilic phases, possess limited water solubility. Altering water solubility of given agents by the addition of trimethylammonium moieties may be used to enhance transport processes.

Binding of Alkyl Ammonium Ions, Neurotransmitters and Neurotransmitter Transport

Having demonstrated that anionic calixarenes function as potent transporters of choline conjugates, we became interested in binding and transport of biologically relevant amines such as serotonin and dopamine. These guests include an endogenous ammonium group. Indeed calixarenes can sequester organic ammoniums via cation- π interactions,^[3] calix[5]arenes and calixarene pentesters with C_{5v} cone conformations are noteworthy in this regard.^[22,23] Calix[6]arene esters also display this behavior.^[24] Hence, calix[5]arene ether and ester derivatives **11** and **12** were added to this study as potential transporters of neurotransmitters **13** and **14** (Figure 2).

We first confirmed the pH sensitive complexation and decomplexation of host **11** with *n*-octylamine (Supporting Information). The ^1H NMR of **11** and *n*-octylamine did not show any evidence of guest binding, but dramatic changes were observed in the upfield region upon protonation of the amine following addition of CF_3COOD . Based on integration and a literature report^[22] a 1:1 inclusion complex is evident. Decomplexation occurred upon addition of diisopropylamine and this cycle could be repeated (Supporting Information). From these observations it was clear that calix[5]arene derivatives can effectively bind primary ammonium ions and release the bound ammonium ion as a function of pH, in an NMR tube.

We then turned our attention to the neurotransmitters dopamine **13** and serotonin **14**, both possess endogenous ammoniums. Unfortunately, calix[5]arene benzyloxy ether **11**, which was a good receptor of *n*-octylammonium trifluoroacetate, did not show any evidence of binding to dopamine **13** or serotonin **14** hydrochlorides by means of ^1H NMR spectroscopy. This was somewhat surprising since isomeric butylamines showed some affinity for similar calix[5]arene ethers.^[22] It is likely that the larger aromatic groups of **13** and **14** provide too much of a steric burden. Calix[5]arene pentaester **12**, on the other hand, displayed strong

binding affinity with both ammonium salts due to formation of inclusion complexes as evidenced by dramatic upfield shifting of NMR signals representing the ethylene protons of both amine hydrochlorides (Supporting Information). No NMR evidence of binding of **13** or **14** with calix[4]arene tetraphosphonic acid **1** was observed in a variety of solvent systems. Nevertheless, we included ionizable calix[*n*]arenes alongside pentaether **11** and pentaester **12** in our transport studies.

When dopamine **13** was subjected to three-phase transport vs. calixarene receptors, we found many favorable combinations that exceed transport levels in the absence of host. Despite having no clear NMR-based evidence of binding, calix[4]arene phosphonic acid **1** transported 22 times more dopamine than was observed in control experiments under neutral receiving phase conditions (Table 5). Acidification of the receiving phase attenuated this effect, yet a 13-fold enhancement in transport was still achieved. Calix[4]arene carboxylic acid **2** induced a 2-fold enhancement in transport, whereas the larger calix[5]arene carboxylic acid **3** enabled a 9-fold enhancement under neutral conditions. The non-ionizable receptors **11** and **12**, (the latter a potent binder as observed by NMR) failed to effect transport, although **12** appeared to serve as an extractor of **13** since transport was 0.8 times that of the appropriate control systems.

Serotonin **14** presented a different story; no background transport was measurable and thus comparisons of enhancements were not possible. Nevertheless we found that **1** was the most potent transporter of **14** as measured by transport flux alone (7.73×10^{-5} , Table 6) and that **1** is also a transporter of dopamine with a similar degree of efficiency (1.18×10^{-4} , Table 5). Of the carboxylic acids **2** and **3**, the latter displayed greater potential. Relative to the lowest transport levels in this series of hosts and receiving phases, phosphonic acid **1** under neutral conditions and calix[5]arene carboxylic acid **3** appear to be superior. The non-ionizable receptors **11** and **12** again failed to compete with **1–3**.

Transport of Ammonium Carboxylate-Conjugates in Water and Buffer

In addition to the trimethylammonium and ammonium handles, we identified one final host complementary handle in our studies. We originally envisioned calcein **15** (Figure 3) as a control molecule for ongoing work we are doing in vesicle systems. Indeed, our original work on transport unveiled very different behaviors with carboxyfluorescein (no transport) versus FITC–choline (transport).^[1] We observed some transport in vesicle systems for calcein. Lacking a trimethylammonium, or ammonium group we carried out three-phase transport experiments as described above and found amplified guest molecule transport in response to the use of host **1**. In water no transport of guest **15** was observed; the addition of **1** afforded a flux of 4.09×10^{-6} ($\mu\text{M cm}^{-2}\text{min}^{-1}$). We first noted this result under buffered conditions and when the source and receiving phase were changed from water to 10 mM HEPES Buffer (pH 7.4) transport of **15** in the absence of host was observed (2.85×10^{-6}); addition of **1** to the organic phase led to a $\approx$ 4-fold increase in transport (1.11×10^{-5}). This is our first report using buffered solutions. These buffered systems more closely approximate those in which we envision ultimate application of the ideas reported herein. Moreover, we propose that the ammonium dicarboxylate group present in **15** serves as a third handle for receptor **1**. Work in both buffer and with this new handle is underway.

Conclusions

We have successfully demonstrated a variety of calixarene-mediated choline-drug conjugate and neurotransmitter transport processes. The key feature is a receptor complementary handle. Choline serves this role well for a variety of phosphonic acid and carboxylic acid functionalized receptors. Previously, we had shown that choline-fluorescein and choline-NBD dye conjugates were transported across a three-phase liquid membrane system with significant enhancements in the presence of calixarenes **1**, **2**, and **4**.^[1] The parent compounds carboxyfluorescein and NBD chloride, both lacking receptor complementary choline handles failed to undergo such transport. We now show that this strategy extends beyond fluorophores; an amino acid, acetaminophen, ibuprofen and bentiromide also are amenable to such transport processes. The neurotransmitters dopamine and serotonin with ionizable amines work as well. We had very modest results with dimethylresveratrol–choline and tamoxifen–choline resisted transport altogether. We find that these more lipophilic substrates present a considerable challenge to our transport system – they partition into a lipophilic environment and calixarenes can even enhance this extraction event. Can we develop a strategy to release lipophilic substrates? We hope that a new handle or host will evolve in answer to this challenge.

We now know that NMR is not powerful as a predictive tool for transport – indeed non-ionizable calixarene ether **11** and ester **12** serve as hosts for neurotransmitters, but do not serve as effective transporters. Acids **1–3** serve as transporters for **13–14**, but not as hosts (via NMR). This realization will, no doubt, be important to the development of future systems – *weak binders should not be ruled out as possible transporters*.

The correlation between extraction and transport is less clear-cut but some testable behaviors emerged. Most notable was the comparison between acetaminophen **6** and ibuprofen **7**. Compound **6** is more hydrophilic and we found that calix[4]arene carboxylic acid was the superior transporter for **6** under acidic receiving phase conditions. Conversely, the slightly more lipophilic **7** was moderately more amenable to transport by the calix[4]arene phosphonic acid **1**.

Looking forward, this strategy provides new ideas and approaches to the drug transport problem. We hope to demonstrate that a drug can be appended to a receptor complementary handle and that the receptor, once lodged in a cell membrane, can serve as a selective shuttle. Moreover, we aim to transport multiple complementary guests per receptor molecule. This strategy diverges from emulsification or covalent conjugate strategies yet is not so farfetched as to prohibit ultimate applications in cells.^[11,25] We know that supramolecular receptors are compatible with cells and we can monitor their localization tendencies in giant unilamellar vesicles.^[9] A final report on the behavior of guests in buffer as well as a comparison of trimethylammonium vs. ammonium vs. ammonium dicarboxylate species will be reported in due course. Our first report using vesicles to visualize and quantitate transport will follow shortly thereafter.

Experimental Section

General

All reagents were of reagent grade or better and used without further purification unless otherwise noted. NMR were acquired with Bruker Fourier 300-MHz or Bruker Avance III 400-MHz spectrometers. Spectra were processed (iNMR) using a Fourier transform with exponential weighting. NMR solvents were purchased from Cambridge Isotope Laboratories and residual solvent peaks were used as internal standards. SiliaFlash® P60 Silica Gel, 40–63 μm , 60A was purchased from Silicycle [Quebec City (Quebec), CA]. Mass analyses were conducted at the University of California, Riverside High Resolution Mass Spectrometry Facility, Riverside, CA, USA. See Supporting Information for full synthetic procedures and spectroscopic data of new compounds.

Extraction Experiments

Two-phase extraction experiments were carried out in 20 mL glass vials. The organic phase consisted of a solution of 0.5 mM for calixarene-based receptors in DCM and the aqueous phase consisted of a solution of **5** in milliQ water. The substrate concentration was varied from 0.1 mM to 3.0 mM. Equal volumes (2 mL each) of organic and aqueous phases were mixed in the vial and the mixture was stirred at 160 rpm for 24 h. After cessation of stirring and phase separation, samples from aqueous phase were analyzed with UV/Vis spectrophotometer to determine the fraction transported in organic phase. The experiments were conducted in duplicate and the results presented are averages. Control experiments were performed without the host.

U-Tube Transport Experiments

Three-phase U-tube transport experiments were conducted by using an U-shaped glass tube as shown below (Figure 4). The organic phase consisted of 10 mL solution 0.5 mM calixarene-based receptors in DCM. The source phase consisted of 4 mL aqueous solution of a substrate in MilliQ water. The receiving phase was either pure milliQ water, 0.1 M HCl or 10 mM HEPES buffer. The U-tube's upper ends were sealed and the experiments were started by stirring the organic phase with magnetic stirring bar being rotated at 400 rpm. The samples from source phase as well as receiving phase were drawn after 72 h and analyzed with UV/Vis spectrophotometer after adequate dilution with milliQ water against a standard serial dilution curve. Two sets of experiments were conducted and the data presented are the average values. Control experiments were performed using pure DCM as the organic phase.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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19. Compound **8** was insoluble at 0.1 mM in water. A saturated solution was prepared, filtered and we estimated the concentration using a standard curve for **8** that was obtained using chloroform. No significant differences were observed in the UV spectra of compound **8** in water vs. chloroform. Nevertheless 0.063 mM remains an estimate for the solubility of compound **8** in water.
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Figure 1.
Structure of calixarene-based receptors **1–4** and drug conjugate substrates **5–10**.

Figure 2.
Structure of calixarene ether **11** and ester **12** receptors and guests: dopamine **13**, serotonin **14**.

Figure 3.
Structure of calcein **15**.

Figure 4.
U-tube dimensions.

Table 1Two-phase solvent extraction of tryptophan–choline **5** with different receptors.^[a]

Receptor (0.5 mM)	[5] [mM]	% Extraction	% Extraction control	Extraction efficiency ^[b]
1	0.1	0 ^[c]	6.32	0
	0.2	0 ^[c]	3.91	0
	0.5	0 ^[c]	1.85	0
	1.0	4.03 ± 0.16	1.52	5.02
	3.0	5.70 ± 0.54	1.14	27.4
2	0.1	19.92 ± 0.38	6.32	2.72
	0.2	19.37 ± 1.30	3.91	6.18
	0.5	13.83 ± 1.01	1.85	11.98
	1.0	13.21 ± 0.13	1.52	23.38
	3.0	8.00 ± 0.35	1.14	41.2
4	0.1	68.42 ± 2.25	6.32	12.4
	0.2	54.60 ± 2.87	3.91	20.28
	0.5	48.14 ± 2.50	1.85	46.29
	1.0	27.27 ± 0.50	1.52	51.5
	3.0	19.20 ± 0.76	1.14	108.4 ^[d]

^[a] Receptor concentration 0.5 mM in DCM, extraction % measured from an aqueous solution of guest after 24 h by UV/Vis measurement at 280 nm against a calibration curve, reported as duplicate runs with ± maximum deviation from the mean.

^[b] (Extraction – control) * ([guest]/[host]).

^[c] The absorbance was slightly higher than at time = 0, we believe this is due to low efficiency guest extraction and some host extraction into the aqueous layer.

^[d] “100%” with error.

Table 2Three-phase transport of tryptophan–choline **5** with different receptors.^[a]

Receptor (0.5 mM)	[5] [mM]	Transport flux ($\mu\text{mol cm}^{-2}\text{min}^{-1}$)	Enhancement ^[b]	Receiving phase
1	0.5	5.76×10^{-5} $\pm 0.09 \times 10^{-5}$	4.5	neutral
	5.0	4.50×10^{-4} $\pm 0.05 \times 10^{-4}$	1.5	
2	0.5	1.18×10^{-5} $\pm 0.00 \times 10^{-5}$	0.9	
	5.0	2.32×10^{-4} $\pm 0.11 \times 10^{-4}$	0.8	
3	0.5	3.03×10^{-6} $\pm 0.00 \times 10^{-6}$	0.2	
	5.0	1.34×10^{-4} $\pm 0.37 \times 10^{-4}$	0.4	
4	0.5	0	0	
	5.0	2.27×10^{-4} $\pm 0.29 \times 10^{-4}$	0.7	
Control (no host)	0.5	1.27×10^{-5}		
	5.0	3.05×10^{-4}		
1	0.5	1.13×10^{-4} $\pm 0.02 \times 10^{-4}$	6.3	acidic (0.1 M HCl)
	5.0	6.76×10^{-4} $\pm 0.02 \times 10^{-4}$	1.9	
2	0.5	1.66×10^{-4}	9.2	
	5.0	$\pm 0.07 \times 10^{-4}$ 9.62×10^{-4} $\pm 0.09 \times 10^{-4}$	2.8	
3	0.5	1.71×10^{-4} $\pm 0.18 \times 10^{-4}$	9.6	
	5.0	1.20×10^{-3} $\pm 0.15 \times 10^{-3}$	3.4	
4	0.5	1.18×10^{-4} $\pm 0.07 \times 10^{-4}$	6.6	
	5.0	6.71×10^{-4} $\pm 0.01 \times 10^{-4}$	1.9	
Control (no host)	0.5	1.79×10^{-5}		
	5.0	3.48×10^{-4}		

^[a]Transport flux measured via U-tube three-phase apparatus: 0.5 mM receptor in 10 mL DCM, the substrate was charged in the source phase at the indicated concentration in 4 mL of water and a receiving phase of 4 mL of water or 0.1 M HCl was added. After 72 h of stirring at 400 rpm, an

aliquot from the receiving phase was removed and analyzed by UV/Vis measurement at 280 nm against a calibration curve, reported as duplicate runs with $\pm$ maximum deviation from the mean.

^[b] Enhancement: flux/flux of corresponding control.

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Table 3Three-phase transport of drug-conjugates acetaminophen–choline **6** and ibuprofen–choline **7**.^[a]

Receptor (0.5 mM)	Guest (0.5 mM)	Transport flux (umol cm ⁻² min ⁻¹)	Enhancement ^[b]	Receiving phase
1	6	5.42×10 ⁻⁶ ± 0.09 × 10 ⁻⁶	2.9	neutral
		1.60 × 10 ⁻⁶ ± 0.12× 10 ⁻⁶	0.8	acidic
2	6	4.25 × 10 ⁻⁶ ± 0.36 × 10 ⁻⁶	2.2	neutral
		1.17 × 10 ⁻⁵ ± 0.15 × 10 ⁻⁵	6.0	acidic
Control (no host)	6	1.89 × 10 ⁻⁶		neutral
		1.96 × 10 ⁻⁶		acidic
1	7	0.00	0	neutral
		1.32 × 10 ⁻⁴ ± 0.10× 10 ⁻⁴	5.6	acidic
2	7	0.00	0	neutral
		7.08 × 10 ⁻⁵ ± 0.20 × 10 ⁻⁵	3.0	acidic
Control (no host)	7	2.37 × 10 ⁻⁵		neutral
		2.37 × 10 ⁻⁵		acidic

^[a]Transport flux measured via U-tube three-phase apparatus: 0.5 mM receptor in 10 mL DCM, 0.5 mM substrate was charged in the source phase in 4 mL of water and a receiving phase of 4 mL of water or 0.1 M HCl was added. After 72 h of stirring at 400 rpm an aliquot from the receiving phase was removed and analyzed by UV/Vis measurement at the corresponding absorbance maximum against a calibration curve, reported as duplicate runs with ± maximum deviation from the mean.

^[b]Enhancement: flux/flux of corresponding control.

Table 4Three-phase transport of drug-conjugates dimethylresvera-trol–choline **9** and bentiromide–choline **10**.^[a]

Receptor (0.5 mM)	Guest (0.5 mM)	Transport flux ($\mu\text{mol cm}^{-2}\text{min}^{-1}$)	Enhancement ^[b]	Receiving phase
1	9	8.32×10^{-6} $\pm 0.27 \times 10^{-6}$	0.4	neutral
		1.40×10^{-5} $\pm 0.06 \times 10^{-5}$	3.4	acidic
2	9	2.38×10^{-6} $\pm 0.62 \times 10^{-6}$	0.1	neutral
		1.07×10^{-5} $\pm 0.11 \times 10^{-5}$	2.6	acidic
Control (no host)	9	2.25×10^{-5}		neutral
		4.12×10^{-6}		acidic
1	10	2.27×10^{-5} $\pm 0.53 \times 10^{-5}$ ^[c]	7.0	neutral
		2.16×10^{-5} $\pm 0.32 \times 10^{-5}$	4.0	acidic
2	10	3.06×10^{-5} $\pm 0.80 \times 10^{-5}$	5.7	acidic
Control (no host)	10	3.23×10^{-6}		neutral
		5.36×10^{-6}		acidic
1 ^[d]	bentiromide	4.70×10^{-5} $\pm 0.81 \times 10^{-5}$	1.4 ^[d]	neutral
Control ^[d]	bentiromide	3.22×10^{-5} $\pm 0.64 \times 10^{-5}$		acidic

^[a]Transport flux measured via U-tube three-phase apparatus: 0.5 mM receptor in 10 mL DCM, 0.5 mM substrate was charged in the source phase in 4 mL of water and a receiving phase of 4 mL of water or 0.1 M HCl was added. After 72 h of stirring at 400 rpm an aliquot from the receiving phase was removed and analyzed by UV/Vis measurement at the corresponding absorbance maximum against a calibration curve, reported as duplicate runs with $\pm$ maximum deviation from the mean.

^[b]Enhancement: flux/flux of corresponding control.

^[c]Reported as triplicate runs with $\pm$ S.D.

^[d]At the source – organic interface, a thin layer of precipitate formed and we measured a significant decrease in concentration of the source phase (up to 50%) without an equal increase in the receiving phase.

Table 5Three-phase transport of dopamine **13**.^[a]

Receptor (0.5 mm)	Guest (5.0 mm)	Transport flux ($\mu\text{mol cm}^{-2} \text{ min}^{-1}$)	Enhancement ^[b]	Receiving phase
1	13	1.18×10^{-4} $\pm 0.02 \times 10^{-4}$	22.1	neutral
		6.38×10^{-5} $\pm 0.41 \times 10^{-5}$	13.1	acidic
2	13	9.91×10^{-6} $\pm 1.12 \times 10^{-6}$	1.9	neutral
		9.75×10^{-6} $\pm 0.25 \times 10^{-6}$	2.0	acidic
3	13	4.80×10^{-5} $\pm 0.33 \times 10^{-5}$	9.0	neutral
		1.34×10^{-5} $\pm 0.27 \times 10^{-5}$	2.9	acidic
11	13	0.00	0	neutral
12	13	4.21×10^{-6} $\pm 0.02 \times 10^{-6}$	0.8	neutral
Control (no host)	13	5.34×10^{-6}		neutral
		4.87×10^{-6}		acidic

^[a]Transport flux measured via U-tube three-phase apparatus: 0.5 mm receptor in 10 mL DCM, 5.0 mm substrate was charged in the source phase in 4 mL of water and a receiving phase of 4 mL of water or 0.1 M HCl was added. After 72 h of stirring at 400 rpm an aliquot from the receiving phase was removed and analyzed by UV/Vis measurement at the corresponding absorbance maximum against a calibration curve, reported as duplicate runs with $\pm$ maximum deviation from the mean.

^[b]Enhancement: flux/flux of corresponding control.

Table 6Three-phase transport of serotonin **14**.^[a]

Receptor (0.5 mm)	Guest (5.0 mm)	Transport flux ($\mu\text{mol cm}^{-2} \text{ min}^{-1}$)	Relative transport ^[b]	Receiving phase
1	14	7.73×10^{-5} $\pm 0.10 \times 10^{-5}$	13.6	neutral
		5.03×10^{-5} $\pm 0.84 \times 10^{-5}$	8.9	acidic
2	14	1.83×10^{-5} $\pm 0.23 \times 10^{-5}$	3.2	neutral
		5.67×10^{-6} $\pm 0.43 \times 10^{-6}$	1.0	acidic
3	14	2.59×10^{-5} $\pm 0.02 \times 10^{-5}$	4.6	neutral
		5.22×10^{-5} $\pm 0.40 \times 10^{-5}$	9.2	acidic
11	14	0.00		neutral
12	14	6.06×10^{-7} $\pm 1.38 \times 10^{-7}$		neutral
Control (no host)	14	0.00		neutral
		0.00		acidic

^[a]Transport flux measured via U-tube three-phase apparatus: 0.5 mm receptor in 10 mL DCM, 5.0 mm substrate was charged in the source phase in 4 mL of water and a receiving phase of 4 mL of water or 0.1 M HCl was added. After 72 h of stirring at 400 rpm an aliquot from the receiving phase was removed and analyzed by UV/Vis measurement at the corresponding absorbance maximum against a calibration curve, reported as duplicate runs with $\pm$ maximum deviation from the mean.

^[b]Enhancement could not be determined due to lack of transport in the absence of host; we report relative transport compared to all ionizable hosts (**1–3**).