Enantioselective Transport by a Steroidal Guanidinium Receptor

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Abstract: The cationic steroidal receptors **9** and **11** have been synthesized from cholic acid **3**. Receptor **9** extracts *N*-acetyl-α-amino acids from aqueous media into chloroform with enantioselectivities (L:D) of 7–10:1. The lipophilic variant **11** has been employed for the enantioselective transport of *N*-acetylphenylalanine, a) through dichloromethane (DCM) and dichloroethane (DCE) bulk liquid membranes (U-tube apparatus), and b) through 2.5% (v/v) octanol/hexane via hollow fibre membrane contactors. Significant enantioselectivities and multiple turnovers were observed for both types of apparatus.

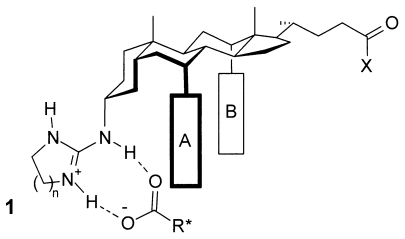
Keywords: chiral resolution · enantioselectivity · membranes · receptors · transport

Introduction

The design of enantioselective receptors is one of the central challenges of supramolecular chemistry.[1] Interest derives partly from the importance of enantioselective recognition in biology, but also from the need for enantiomerically pure compounds in the chemical industry.[2] Indeed, to quote from a recent monograph, "The separation of enantiomers may well represent the economically most important application of supramolecular chemistry".[3] However, to fulfil this potential it is necessary to develop practical receptor-mediated separation procedures. A key issue is that of receptor recycling. A sophisticated receptor is only viable if it can be used many times over, ideally in a continuous process. Membrane transport fulfils this criterion.[4] Receptors may be located in a bulk liquid membrane,[5] or dissolved[6] or covalently incorporated[7] in polymeric membranes. Acting as shuttles, the receptor molecules can transfer many equivalents of substrate between reservoirs. Selectivities depend on the rates of uptake, diffusion and delivery of the two enantiomers, but often reflect the ratio of binding affinities.[5a]

Chiral carboxylates are good subjects for membrane transport because they are soluble in water as alkali metal salts but readily extracted into non-polar media by lipophilic cations. Enantioselective extractions have been accomplished with chiral cations,[5b, 8] and also with achiral cations accompanied by electroneutral chiral receptors.[9] Guanidinium cations are especially suitable, as they are able to form well-defined salt bridges with carboxylates through formation of two parallel hydrogen bonds.[10]

Steroidal guanidinium cations of general form **1** represent a promising new class of enantioselective carboxylate receptors.



1 **2**

Accessible from inexpensive cholic acid **3**, they create a tunable chiral environment for bound carboxylates **2**. Early examples such as **4** and **5** have proved effective, performing extractions with enantioselectivities of 7–10:1 on a range of amino acid derivatives **6**.[11] Herein we report that they also

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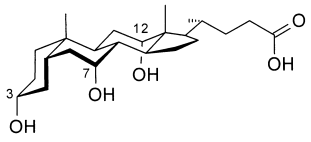

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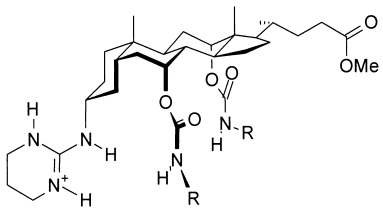
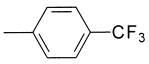
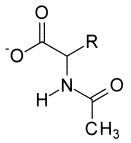


4 R = Ph

5 R = 



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succeed as enantioselective membrane transport agents, both in a U-tube apparatus (bulk DCE/DCM membrane) and also in a system based on hollow fibre membranes. The latter is of particular interest in view of a possible industrial-scale separation method.

Results and Discussion

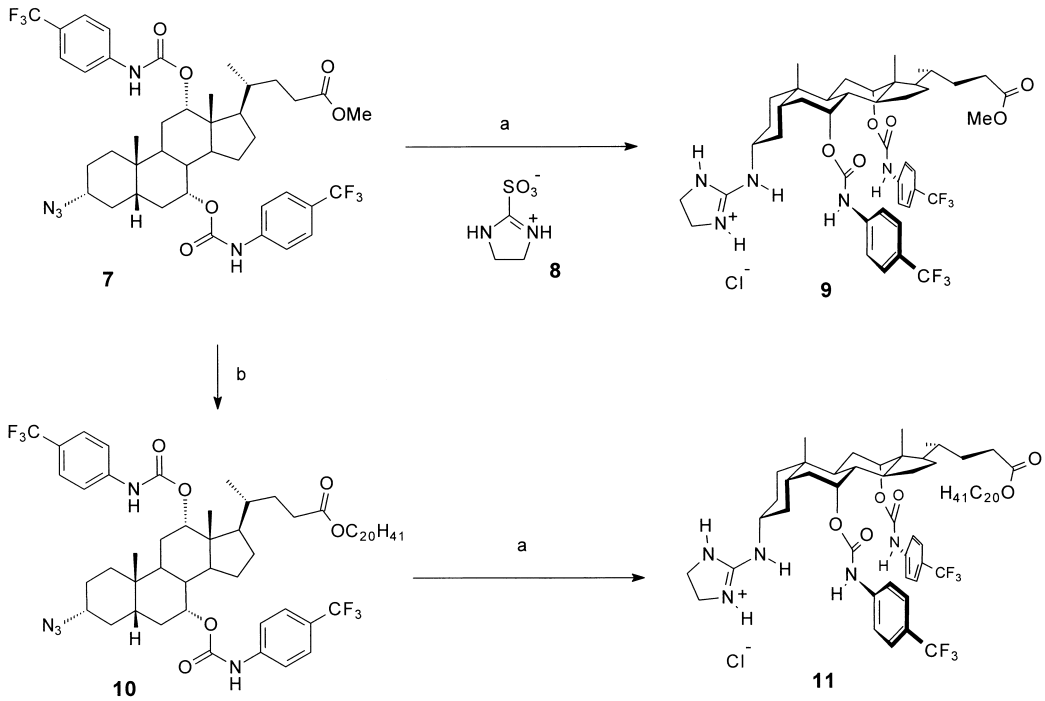
Receptor design and synthesis: The transport experiments described herein required a variant on structure **1** which was a) enantioselective, b) available in gram quantities, and c) highly lipophilic, and soluble in non-polar solvents. The design evolved from **5**, the most successful of our previous systems.[11b] Two changes were made. Firstly, for synthetic reasons, the six-membered ring guanidinium moiety in **5** was replaced by a five-membered unit, as in **9**. As shown in Scheme 1, **9** could be prepared in two steps from azide **7**[11b] by employing the guanylation reagent **8**.[12] In contrast the six-membered unit in **5** had required a low-yielding multistep procedure, as conditions for direct introduction could not be found.[13] Amino acid extraction studies using **9** confirmed that the change in ring size did not greatly affect the enantioselectivity (Table 1).

Table 1. Enantioselective extraction of *N*-acetyl-α-amino acids from aqueous buffer into $CHCl_3$ by receptor **9**.[a]

Substrate	Extraction efficiency [%][b]	Enantioselectivity (L:D)[c]
N-Ac-DL-alanine	77	10:1
N-Ac-DL-valine	81	7:1
N-Ac-DL-phenylalanine	87	10:1

[a] For experimental details, see ref. [11b]. [b] Concentration of substrate in organic phase, as percentage of receptor concentration, determined by NMR integration. [c] Determined by NMR integration of signals for complexed substrates in extracts.

Secondly, extraction experiments involving methyl esters such as **4**, **5** or **9** were generally found to result in the loss of significant amounts of receptor from the organic phase. Moreover, **9** was found to be insoluble in the alkane/octanol mixtures used with the hollow fibre membrane system (vide infra). The methyl ester was therefore changed to an eicosyl ester, solving both problems. As shown in Scheme 1, azido ester **7** was treated with aqueous sodium hydroxide, followed by caesium carbonate and eicosyl bromide, to give eicosyl



Scheme 1. Synthesis of receptors **9** and **11**: a) 1) Zn, AcOH, 2) **8**, $NaHCO_3$, DMF, 3) CH_2Cl_2, 1M NaOH aq. then 0.5M HCl aq.; b) 1) NaOH, MeOH, H_2O, 2) HCl aq., 3) $C_{20}H_{41}Br$, Cs_2CO_3, NaI (cat.), DMF.

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ester **10**. Reduction of the azide followed by treatment with **8** gave receptor **11**, which was used in the transport experiments described below.

Transport by receptor 11 through dichloromethane (DCE)/ dichloroethane (DCM) membranes (U-tube apparatus): Transport through chlorinated solvent membranes was investigated in the U-tube apparatus depicted in Figure 1. Based on previous extraction experiments, *N*-Ac-L/D-PheOH were selected as substrates. Initial experiments were performed on

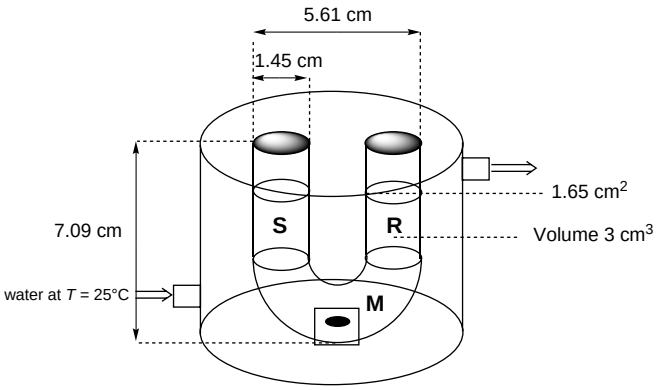

Figure 1. Transport cell, with dimensions.

the individual enantiomers, exploring the effect of various parameters that could affect transport rate. The source phase consisted of *N*-Ac-L-PheOH or *N*-Ac-D-PheOH dissolved in TRIS buffer (pH = 8.1). The organic (membrane) phase consisted of a solution of **11** in 1,2-dichloroethane, and the receiving phase was aqueous KBr. Bromide was found to be more effective than chloride for counter-current exchange at the membrane-receiving phase boundary. Concentrations of KBr were higher than those of substrate, to ensure efficient active transport. Transport rates varied linearly with [**11**] at low to moderate receptor concentrations, but reached a plateau at concentrations of [**11**] of about 3.4×10^{-2} M. A temperature increase from 25 °C to 40 °C caused no significant changes in the transport rate. The relative transport rates measured for the two enantiomers were consistent with the extraction experiments, in that the L enantiomer was transported at least four times faster than the D enantiomer (Figure 2).

Carrier **11** was also tested for transport of racemic *N*-acetylphenylalanine in the U-tube apparatus. Conditions were similar to those above described for the single enantiomers, except that dichloromethane was employed as the bulk membrane phase instead of 1,2-dichloroethane. The results are summarized in Table 2. Notable selectivity was shown for *N*-Ac-L-PheOH. A consistent value of nearly 70% *ee* was found at the initial stages of the experiment, the figure only decreasing significantly after 24 h, when the source phase become increasingly concentrated in the other enantiomer (Figure 3). Over the course of the experiment, about 20 equivalents of *N*-acetylphenylalanine was transported by receptor **11**.

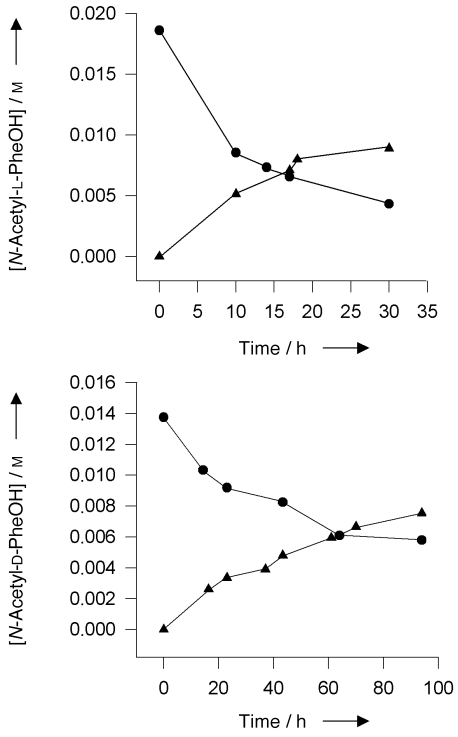

Figure 2. Active transport of *N*-Ac-PheOH single enantiomers with carrier **11** in U-tube apparatus (Figure 1). Triangles represent receiving phases and circles source phases

Table 2. Enantioselective transport of *N*-Ac-PheOH in a U-tube using **11** as carrier.

Time [h]	*N*-Ac-Phe [%]	*ee* [%]
2	3	66
3	5	71
4	6	70
5	7.5	64
6	8	68
7	12	64
8	10	64
9	12	60
24	27	56

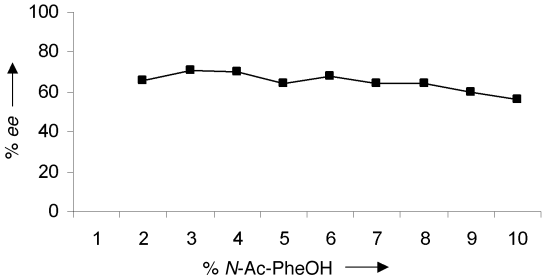

Figure 3. Enantioselective transport of *N*-Ac-DL-PheOH in U-tube apparatus; *ee* in receiving phase is plotted against total transported.

Enantioselective transport in hollow fibre membrane contactors: Although useful in the laboratory, transport in U-tubes does not scale up effectively due to the relative decrease in contact surface area. To investigate its behaviour in an industrially relevant setting, receptor **11** was also tested in the separator depicted in Figure 4 (see also Table 3). The

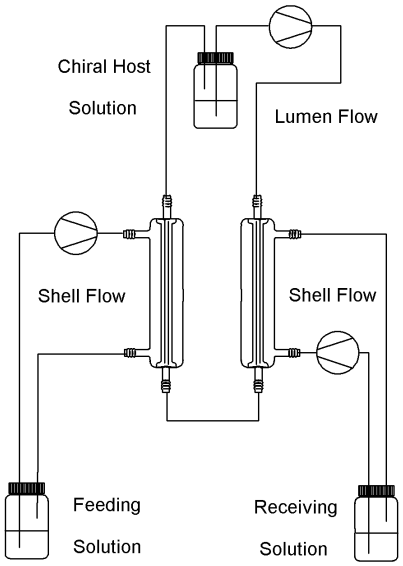

Figure 4. Experimental set-up for simultaneous extraction and stripping of *N*-Ac-DL-PheOH using two hollow fibre contactors.

Table 3. Characteristics of the hollow fibre modules and membrane Microdyn LD OC 02.

Module and membrane data	
diameter	2 cm
length	35 cm
number of fibres	1280
internal diameter, dry	200 μm
wall thickness, dry	9.5 μm
active membrane area, inside	0.2 cm^2
active membrane length	24.5 cm
free flow area	0.4 cm^2
intracapillary volume	11 mL
extracapillary volume	25 mL

key elements of this apparatus are contactor modules incorporating hollow cellulose fibres. A solution of chiral selector in a non-polar, water-immiscible solvent is pumped through the interiors of the fibres (lumen flow), while the aqueous source and receiving phases are in contact with the exteriors. Due to the high surface area, efficient transfer occurs between phases. A similar apparatus was used by Pirkle and co-workers to separate *N*-(3,5-dinitrobenzoyl)leucine enantiomers, with lipophilic (*S*)-*N*-(1-naphthyl)leucine derivatives as selectors.[5d] However, this work used an excess of chiral host, while the present experiments employed **11** in just catalytic amounts. Unfortunately the material of the hollow fibre contactor limits the choice of organic solvent for the lumen flow; only aliphatic hydrocarbons and alcohols are compatible. 2.5% (v/v) 1-octanol in hexane was chosen for this work, being sufficiently polar to dissolve **11** but incapable of supporting transport in the absence of receptor (as shown by control experiments).

The separation of *N*-Ac-DL-PheOH in the membrane unit was performed as follows: A source phase consisting of 5 g of racemic *N*-Ac-PheOH in 500 mL of TRIS buffer was circulated through the shell of one module, while a receiving phase of 0.1M KBr in 500 mL of TRIS buffer was circulated through the shell of the other. Through the lumen of both modules was circulated an organic phase consisting of octanol/hexane 2.5% (v/v) (125 mL), 4 mM in host **11**. The concentrations of the enantiomers in each aqueous phase and the *ee* values were monitored over 48 h (Figure 5). After this time, the amino acid transported to the receiving phase corresponded to 60% of the initial amount. Initially an *ee* of 31% was observed in the receiving phase, although this was eroded over time as the system moved towards equilibrium (which must, in principle, involve racemates in both source and receiving phases). Up to 70 equivalents of substrate were transported by the receptor.

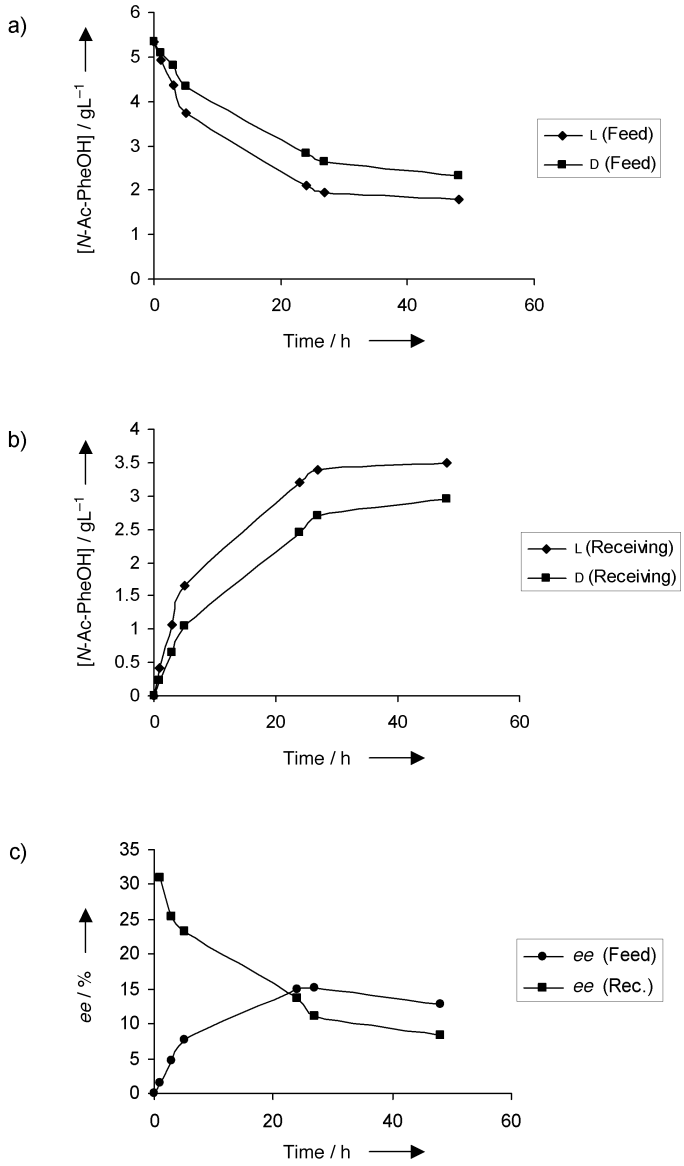

Figure 5. Enantioselective transport of *N*-Ac-DL-PheOH in hollow fibre membrane apparatus. a) Depletion of substrate enantiomers in source phase. b) Accumulation of substrate enantiomers in the receiving phase. c) Enantiomeric excess of N-Ac-PheOH in source and receiving phases.

Transport rates and enantioselectivities were found to be very sensitive towards changes in the source and receiving phases. With phosphate buffer instead of TRIS, the rate of transport was very similar but no enantioselectivity was

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observed. The use of unbuffered 0.1 M KBr in the receiving phase gave good enantioselectivity (33%), but transport leveled off at 12%. A higher concentration of KBr (1 M) in the receiving phase lowered transport rates by a factor of 3, despite the increased thermodynamic driving force. Presumably this results from lowered concentrations of the carboxylate in the organic phase, due to competition by bromide for cationic binding sites. To confirm that the enantioselective transport was due to the chiral host and not to the cellulose in the fibres, we ran an experiment using tetraoctylammonium bromide (TOAB) as carrier. As expected, non-enantioselective transport was observed. The lowered initial selectivity observed in hollow fibre membrane apparatus (ca. 30% *ee*) compared to that for the U-tube (ca. 70% *ee*) was due to the change in solvent. Transport of *N*-Ac-DL-PheOH by **11** through a bulk membrane of 2.5% (v/v) 1-octanol in hexane also occurred with about 30% *ee*.[14]

Conclusion

In conclusion, we have synthesized the lipophilic steroidal guanidinum receptor **11**, and demonstrated its effectiveness as an enantioselective transport agent for an *N*-acetyl-α-aminocarboxylate. In a conventional U-tube apparatus with chlorinated solvents as bulk liquid membranes, the enantioselectivities approached those measured in simple extraction experiments. In a system based on hollow fibre membrane contactors, somewhat lower selectivities were observed due to an enforced change in membrane solvent. In both cases substantial turnover was observed, confirming the ability of **11** to function as a catalytic agent for enantioselective separation. Although selectivities dropped as transport proceeded, this problem can be remedied in a number of ways: for example, a) extraction of the source phase with two independent loops containing hosts with opposite selectivities, b) continuous racemisation of the source phase, and c) the use of several membrane units in series, running the separation in a countercurrent fashion.[4]. We are currently investigating this last option, as it holds promise for a versatile and practical enantioseparation system for development-scale intermediates and products.

Experimental Section

General: ^{1}H and ^{13}C NMR spectra were obtained from a Bruker MSL-300 or a Bruker DPX-400 spectrometer. Chemical shifts are reported relative to residual undeuterated solvent peaks. Melting points were determined on a Gallenkamp melting-point apparatus. Infrared spectra were recorded on a Perkin-Elmer 883 or Perkin-Elmer FT-IR Paragon 1000 spectrophotometer. Reactions were monitored by thin-layer chromatography (TLC) on Merck silica gel 60 F_{254} aluminium-backed plates. Spots due to steroidal compounds were visualised by charring over a Bunsen burner. Flash chromatography was carried out on Merck silica gel 60 (particle size 0.040–0.063 mm). Reagents were purchased from Aldrich, Fluka or Sigma and were used without further purification.

Methyl 3α-(4,5-dihydro-1*H*-imidazol-3-ium-2-yl)-7α,12α-bis[(*p*-trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate chloride (9): Acetic acid (6 mL) was added to a mixture of zinc dust (200 mg, 3.0 mmol) and compound **7**[11b] (100 mg, 0.12 mmol). The reaction mixture was protected from the atmosphere by a calcium chloride tube and stirred vigorously at room temperature for 45 min. The zinc residues were removed by filtration and washed with acetic acid (3×5 mL). The filtrate was evaporated under reduced pressure. Residual acetic acid was removed by repetitive addition and evaporation under reduced pressure of distilled toluene (2×5 mL) to afford the crude alkylammonium acetate (99 mg, 95%). Guanylating reagent **8**[12] (29 mg, 0.19 mmol) was added to a well-stirred suspension of the alkylammonium acetate (50 mg, 58 μmol) and $NaHCO_3$ (60 mg, 0.72 mmol) in DMF (625 μL) at 80 °C. The mixture was vigorously stirred for 1 h then allowed to cool to room temperature and evaporated under reduced pressure. The residue was purified by flash chromatography eluting with EtOAc. To ensure chloride was the counter anion, the resulting purified salt was taken up in CH_2Cl_2 (5 mL) and washed carefully with aqueous NaOH (1 M, 2×5 mL) followed by triply distilled water (5 mL). The organic layer was then washed with aqueous HCl (0.5 M, 2×5 mL) followed by triply distilled water (2×5 mL). The organic phase was then dried by decanting and evaporated under reduced pressure. The residue was then further dried in vacuo at 60 °C to afford the pure guanidinium chloride **9** (36 mg, 72%) as a white solid; $R_f = 0.69$ (EtOAc); m.p. 195–198 °C; ^{1}H NMR (400 MHz; $CDCl_3$): $\delta = 0.76$ (s, 3H; 18-CH_3), 0.86 (d, $^3J(H,H) = 6.5$ Hz, 3H; 21-CH_3), 0.94 (s, 3H; 19-CH_3), 3.04 (br m, 1H; 3β-H), 3.64 (s, 3H; CO_2CH_3), 3.73 (s, 4H; CH_2's at positions 4 and 5 of the imidazolinium ring), 4.89 (s, 1H; 12β-H), 5.19 (s, 1H; 7β-H), 5.61 (br s, 1H; NH at position 1 or 3 of the imidazolinium ring), 6.75 (br s, 1H; NH at position 1 or 3 of the imidazolinium ring), 7.49 (d, $^3J(H,H) = 7.9$ Hz, 4H; ArH *meta*), 7.94 (m, 4H; ArH *ortho*), 9.05 (s, 1H; NHCO), 9.59 (br s, 1H; 3α-NH), 9.69 (s, 1H; NHCO); ^{13}C NMR (100 MHz; $CDCl_3$): $\delta = 11.50$ (18-CH_3), 16.65 (21-CH_3), 21.30 (19-CH_3), 21.84 (CH_2), 25.07 (CH_2), 26.07 (C-3 or 4 of imidazolium ring), 26.13 (C-3, 4 imidazolium ring), 26.15 (CH_2), 27.78 (CH_2), 29.65 (CH), 29.96 (CH_2), 30.22 (CH_2), 32.77 (10-C), 33.51 (CH_2), 33.67, 33.92, 38.43 (CH), 39.99 (CH), 42.30 (CH), 44.25 (13-C), 46.20 (CH), 50.47 (3-CH), 52.72 (CO_2CH_3), 70.17 (7-CH), 75.69 (12-C), 117.16 (ArCH *ortho*), 117.45 (ArCH *ortho*), 124.70 (ArCH *para*), 124.90 (ArCH *para*), 124.94 (ArCH *meta*), 124.98 (ArCH *meta*), 125.02, 125.05, 141.09, 141.15, 152.21, 152.37 (C), 157.50 (C), 173.70 ($COOCH_3$); FAB-MS *m/z*: 864 $[M + Na]^+$; HRMS (FAB^+) calcd for $C_{44}H_{56}N_5O_6F_6$: 864.413480; found: 864.410431; elemental analysis calcd (%) for $C_{44}H_{56}N_5O_6F_6Cl \cdot 1.5\,H_2O$ C 57.98, H 6.52, N 7.68; found: C 57.98, H 6.76, N 7.47.

Eicosyl 3α-azido-7α,12α-di[*p*-(trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate (10): To a solution of compound **7** (5.00 g, 6.08 mmol) in methanol/water (50 mL, 9:1) was added sodium hydroxide (1.08 g, 27 mmol). The mixture was stirred at room temperature overnight. The solvent was removed under reduced pressure and the white solid was taken up in dichloromethane. Aqueous HCl (1 M) was added until pH 4 was reached. The mixture was extracted with dichloromethane and the organic layer was washed with water, dried and concentrated under reduced pressure. The white solid was dissolved in methanol/water (50 mL, 9:1) and caesium carbonate (0.99 g, 3.04 mmol) was added. The solvents were removed and the solid was taken up in dry DMF (80 mL). Bromoeicosane (2.2 g, 6.08 mmol) and sodium iodide (90 mg, 0.6 mmol) were added and the mixture was stirred under argon for 24 h at 47 °C. The solvent was removed under reduced pressure and the product was isolated by flash chromatography (hexane/ethyl acetate, 5:1) to yield eicosyl ester **10** (5.46 g, 82%). $R_f = 0.81$ (ethyl acetate/hexane/chloroform, 1:1:1); m.p. 165 °C; IR (film from $CDCl_3$): $\tilde{\nu}_{max} = 2090$ cm^{-1} (N_3), 1739 cm^{-1} (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.75$ (s, 3H; 18-CH_3), 0.90 (t, 3H; esterCH_3), 0.94 (d, 3H; 21-CH_3), 0.97 (s, 3H;19-CH_3), 1.25 (m, 38H; side chain CH_2's), 3.18 (m, 1H; 3β-H), 4.01 (t, 2H; CO_2CH_2), 4.97 (m, 1H; 7β-H), 5.13 (m, 1H; 12β-H), 6.94 (br s, 1H; NH), 7.06 (br s, 1H; NH), 7.67 (s, 8H; ArH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.3$, 14.1, 17.6, 22.5, 22.7, 22.9, 25.8, 25.9, 26.8, 27.1, 28.6, 29.1, 29.2, 29.4, 29.5, 29.6, 29.7, 30.7, 31.2, 31.5, 31.9, 34.4, 34.5, 34.7, 35.0, 37.9, 41.1, 43.7, 45.4, 47.5, 61.2, 64.6, 72.4, 118.1, 118.2, 126.4, 141.1, 152.4, 152.7, 174.2; HRMS (ES^+) calcd for $C_{60}H_{87}N_5O_6F_6Na$ $[M + Na]^+$: 1110.6458; found: 1110.6500.

Eicosyl 3α-(4,5-dihydro-1*H*-imidazol-3-ium-2-yl)-7α,12α-bis[(*p*-trifluoromethyl)phenylaminocarbonyloxy]-5β-cholan-24-oate chloride (11): To the azide **10** (3.05 g, 2.8 mmol) in glacial acetic acid (15 mL) was added zinc dust (1.00 g). The mixture was stirred for 8 h at room temperature, after which the mixture was filtered and evaporated to dryness to give the corresponding alkylammonium acetate as an off white solid (2.45 g, 78%). ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.81$ (s, 3H; 18-CH_3), 0.89 (m, 6H; eicosyl

CH_3 and 19-CH_3), 0.95 (d, 3H; 21-CH_3), 2.05 (s, 3H; acetate CH_3), 2.81 (m, 1H; 3β-H), 4.01 (t, 2H; CO_2CH_2), 5.03 (m, 1H; 7β-H), 5.25 (m, 1H; 12β-H), 6.29 (br, 3H; NH_3^+), 7.52 (m, 8H; ArH), 9.37 (s, 1H; 7α-NH), 9.57 (s, 1H; 12α-NH); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.3$, 13.7, 17.3, 21.9, 22.2, 25.5, 26.8, 28.2, 28.8, 29.0, 29.1, 29.3, 30.4, 31.1, 31.5, 33.2, 34.5, 37.2, 45.1, 47.3, 64.1, 117.6, 117.8, 125.7, 126.0, 142.2, 152.7 (NHCO), 153.1 (NHCO), 173.8 (C-24), 178.5 (CH_3CO_2). To a solution of this material (2.4 g, 2.20 mmol) and sodium bicarbonate (2.08 g, 25.2 mmol) in DMF (40 mL) was added reagent **8** (0.94 g, 6.3 mmol). The reaction was stirred for 3 h at 80 °C under argon. The solvents were removed and the product was purified by flash chromatography (dichloromethane/methanol, 20:1). To ensure that chloride was the counter ion, the salt was taken up in dichloromethane and washed with sodium hydroxide (1 M) followed by distilled water. The organic layer was washed with HCl (0.5 M) followed by distilled water. The organic phase was dried and concentrated under reduced pressure to yield **11** (1.47 g, 68%) as a white solid. $R_f = 0.79$ (dichloromethane/methanol, 15:1); m.p. 234–235 °C; IR (film from $CDCl_3$): $\tilde{\nu}_{max} = 1729\ cm^{-1}$ (C=O); ^{1}H NMR (400 MHz, $CDCl_3$): $\delta = 0.77$ (s, 3H; 18-CH_3), 0.87–0.90 (m, 6H; eicosyl CH_3 and 19-CH_3), 0.96 (d, ^3J(H,H) = 6.5 Hz, 3H; 21-CH_3), 3.04 (m, 1H; 3β-H), 3.69 (br s, 4H; $NH(CH_2)_2NH$), 4.00 (t, J(H,H) = 6.8 Hz, 2H; CO_2CH_2), 4.90 (br s, 1H; 7β-H), 5.20 (br s, 1H; 12β-H), 7.49–7.52 (m, 4H; ArH), 7.88–7.95 (m, 4H; ArH), 9.11 (br s , 1H), 9.58 (br s, 1H), 9.68 (br s , 1H); ^{13}C NMR (100 MHz, $CDCl_3$): $\delta = 12.3$ (CH_3), 14.0 (18-CH_3), 17.6 (21-CH_3), 21.7 (19-CH_3), 22.6 (CH_2), 23.8 (CH_2), 25.3 (CH_2), 25.8 (CH_2), 27.0 (CH_2), 27.2 (CH_2), 28.1 (CH), 28.5 (CH_2), (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 29.6 (CH_2), 30.6 (CH_2), 30.9 (CH_2), 31.2 (CH_2), 31.8 (CH_2), 33.4 (C), 34.0 (CH_2), 34.7 (CH), 34.8 (CH_2), 38.0 (CH), 40.6 (CH), 42.7 (CH), 44.9 (C), 47.0 (CH), 53.0 (CH), 64.5 (CH_2), 71.0 (CH), 75.5 (CH), 117.9 and 118.6 (Ar-CH), 123.0, 124.0 and 125.2 (Ar-C), 125.6 and 125.8 (Ar-CH), 142.6 and 142.7 (Ar-C), 153.2 (OCONH), 153.5 (OCONH), 158.3 ($C(NH)_3$), 174.5 (CO_2CH_2); ^{19}F NMR (376 MHz, $CDCl_3$): $\delta = 62.3$; HRMS (FAB$^+$) calcd for $C_{63}H_{94}N_5O_6F_6$ $[M+H]^+$: 1130.7108; found: 1130.7100.

U-Tube transport experiments: *Single enantiomer experiments*: A U-tube glass cell (Figure 1) was employed in all experiments. The temperature was maintained at 25.0 ± 0.1 °C, and the stirring rate was adjusted to 2600 rpm, as measured by means of a tachometer (stirring bar dimensions: $h = 6$ mm, $\varnothing = 2$ mm). *N*-Ac-L-PheOH or *N*-Ac-D-PheOH, (5.0×10^{-2} M) in TRIS buffer (3 mL; pH 8.1, 3 mL) were employed as the source phase. The organic (membrane) phase consisted of a solution of **11** in 1,2-dichloroethane (10 mL; 1.71×10^{-3} M). The receiving phase was a KBr (0.5 M) solution in TRIS buffer (pH 8.1, 3 mL). Amino acid uptake at the source phase and release at the receiving phase was monitored by HPLC (Perkin Elmer Integral 4000, Scharlau LC18 column, UV detector at $\lambda = 230$ nm, injection of 50 µL aliquots taken at one or two hours intervals) using benzyl alcohol as an external standard. A gradient H_2O–CH_3CN (0–100% in 20 minutes), followed by pure CH_3CN (5 min) was employed as eluent. Retention times: *N*-Ac-PheOH $t = 9.1$ min; benzyl alcohol $t = 14.2$ min.

Transport of racemic amino acid: The same U-tube glass cell was employed. The dimensions of the stirring bar for this experiments ($h = 9$ mm, $\varnothing =$ 5 mm, egg shaped) allowed a stirring rate of only 1000 rpm. *N*-Ac-DL-PheOH, (5.0×10^{-2} M) in TRIS buffer (pH 8.1, 3 mL) was employed as the source phase, and the receiving phase consisted of a KBr (0.5 M) solution in TRIS buffer (pH 8.1, 3 mL), as above. A solution of **11** in dichloromethane (8.6×10^{-4} M, 10 mL) was employed as the bulk membrane. The concentrations of the amino acid enantiomers in the receiving phase were monitored by HPLC (Waters 600 Controller, Waters 2487 UV/Vis detector at $\lambda = 254$ nm), employing a chiral column (Chirobiotic T, Astec, USA) under reverse phase conditions (H_2O (1% TEAA)-MeOH, 80:20). Retention times: *N*-Ac-L-PheOH $t = 4.3$ min; *N*-Ac-D-PheOH $t = 6.5$ min. Aliquots of 10 µL from the receiving phase were injected every hour. Calibration was performed injecting 1.1×10^{-1} mg mL^{-1} of a standard solution of *N*-Ac-DL-PheOH.

Transport through hollow fibre membranes: *Materials and apparatus*: The source phase consisted of *N*-Ac-DL-PheOH (4.8×10^{-2} M) in TRIS buffer (4.8×10^{-2} M, pH 8.0 ± 0.1) or phosphate buffer (0.1 M, pH 7.4). The organic phase consisted of a solution of **11** (3–8 mM) in octanol/hexane 2.5% (v/v) (Merck, Germany). The receiving phase was a 0.1–1 M potassium bromide solution in TRIS or phosphate buffer (as above). The system set-up for the simultaneous extraction and stripping process using two hollow fibre contactors is shown in Figure 4. Each hollow fibre module (Dialysis Module type LD OC 02, Microdyn, Wuppertal, Germany) contains 1280 hydrophilic cellulose fibres. The characteristics of the module and fibres are summarised in Table 3. In addition, the housing material is polycarbonate and the potting material (used to secure the fibre bundle to the tube sheet) is polyurethane. The system is driven by three peristaltic pumps (Watson & Marlow 505U) run at 40 rpm.

Separation experiments: The source phase (500 mL) was circulated through the shell of one module while the receiving phase (500 mL) was circulated through the shell of the other (both at 18 mL min^{-1}). Through the lumen of both modules was circulated the organic phase (125 mL, 33 mL min^{-1}). The concentrations of the enantiomers in each aqueous phase were monitored for 48 h using HPLC (Chirobiotic T, Astec, USA; H_2O (1% ammonium acetate)-MeOH, 80:20, pH 4.1). A UV detector (Spectra Physics, USA) at a wavelength of 254 nm was used.

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[13] The six-membered ring analogue of **8** appears to be significantly less reactive, presumably due to steric hindrance and/or ring strain effects.

[14] This experiment was performed in a "W-tube", the organic solvent being less dense than water.

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