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Design, synthesis and membrane ion transport properties of cystine- and serine-based *cyclo*-4-oxa-heptane-1,7-bisamides

Darshan Ranganathan,[a,*] Manoj P. Samant,[a] R. Nagaraj[b] and E. Bikshapathy[b]

[a]*Organic Division III, Discovery Laboratory, Indian Institute of Chemical Technology, Hyderabad 500 007, India*
[b]*Centre for Cellular and Molecular Biology, Hyderabad 500 007, India*

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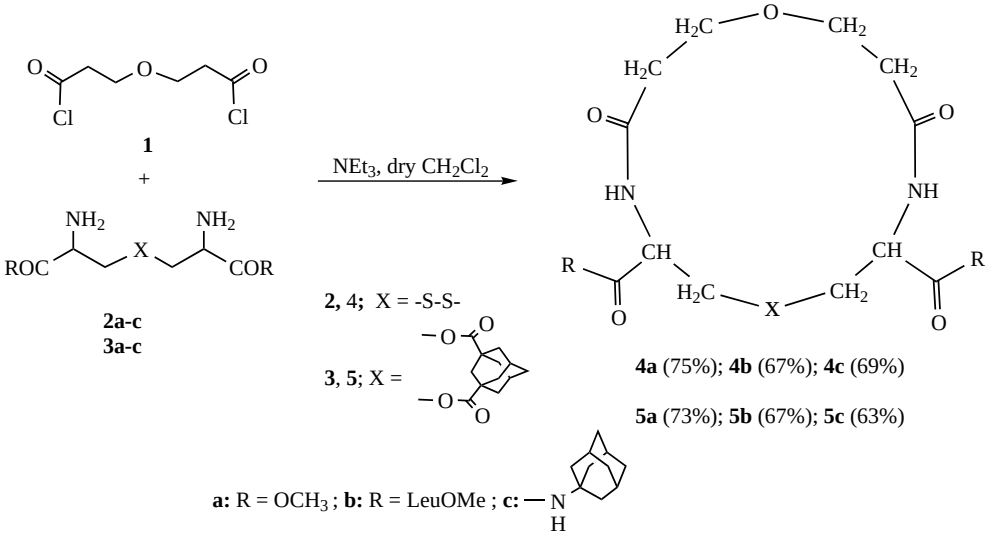
Abstract—A new class of cyclobisamides, having the general profile of amino acid (cystine or serine)–ether composites, as potentially efficient ion transporters has been designed and synthesized. In model membranes, the adamantane harboring composites exhibited 57–66% of gramicidin A activity in ion transport.

The design and synthesis of new macrocycles with tailor-made structural and functional properties continues to attract the attention of synthetic chemists.[1] Our promising efforts in the novel domain of hybrid cyclic peptides[2,3] made it logical to explore systems that are likely to be more effective in ion transport. In this context, cyclic structures that incorporate facets of peptide and ether were considered attractive. This communication reports the design and synthesis of cystine-based 15-membered and serine-based 20-membered *cyclo*-4-oxa-heptane-1,7-bisamides and demonstrates their ability to transport Na^+ ions across model membranes.

The cystine-based 15-membered *cyclo*-4-oxa-heptane-1,7-bisamides (**4a–c**) were prepared by the condensation of **1**[4] with either the simple cystine diOMe (**2a**) or its C,C′-extended bispeptides[5] (**2b–c**) in the presence of NEt_3 under high dilution conditions (Scheme 1). The precursor C,C′-extended bispeptides (**2b–c**) were pre-



Scheme 1.

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* Corresponding author. Fax: +91-40-7160757; e-mail: rangan@iict.ap.nic.in

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pared by DCC/HOSu coupling of bis Boc cystine with either LeuOMe or 1-amino adamantane, followed by N^{α}-Boc deprotection using TFA/CH_2Cl_2.

Similarly, the serine-based 20-membered *cyclo*-4-oxa-heptane-1,7-bisamides (**5a–c**) were prepared by the condensation of **1** with 1,3-adamantane bis-ser depsipeptides (**3a–c**) (Scheme 1). The preparation of 1,3-adamantane bis-ser depsipeptides (**3a–c**) firstly involves the condensation of an *N,C*-protected serine amino acid or its peptide with 1,3-adamantane-dicarbonyl dichloride followed by N^{α}-Z deprotection using 10% Pd/C/H_2.

All cyclobisamides (**4a–c** and **5a–c**) required purification through a short column of silica gel with $CHCl_3$/MeOH as eluent and were fully characterized.[6] In ^{1}H NMR variable-temperature (VT) studies, conducted in DMSO-d_6 between 303 and 343 K, there was no indication of any intramolecular hydrogen bonding of the amide protons as shown by high temperature coefficient values ($d\delta/dT$ >5 ppb K^{-1}).

The ability of cyclobisamides **4a–c** and **5a–c** to transport ions across model membranes (small unilamellar vesicles of palmitoyl oleoyl phosphatidylcholine) was assessed by monitoring the decay of a valinomycin-mediated K^+ diffusion potential using the fluorescent dye method.[7] Of these, **4c** and **5c** were able to dissipate the diffusion potential created by valinomycin (Vm), indicating its ability to transport Na^+ ions across the lipid bilayer (Fig. 1). Compounds **4c** and **5c** were, respectively, assessed as having 57 and 66% of gramicidin A activity, in ion transport. Other compounds in the class exhibited a low profile. None of the cyclobisamides were able to transport Ca^{2+} and Mg^{2+} ions. The compounds **4c** and **5c** did not cause the release of entrapped carboxyflorescein, indicating that the movement of ions across the lipid bilayers was not due to the formation of large pores or detergent-like action.[8] The ion flux may thus be ascribed to a carrier-type of ion-transport mechanism like that of valinomycin.[9]

The design delineated provides a straightforward entry into a new class of amino acid anchored cyclobisamide ionophores. The flexibility in the synthetic strategy with the proper choice of ring-inserts is likely to provide incentives for the design of cyclobisamide ionophores with promise to act as selective hosts in molecular recognition.

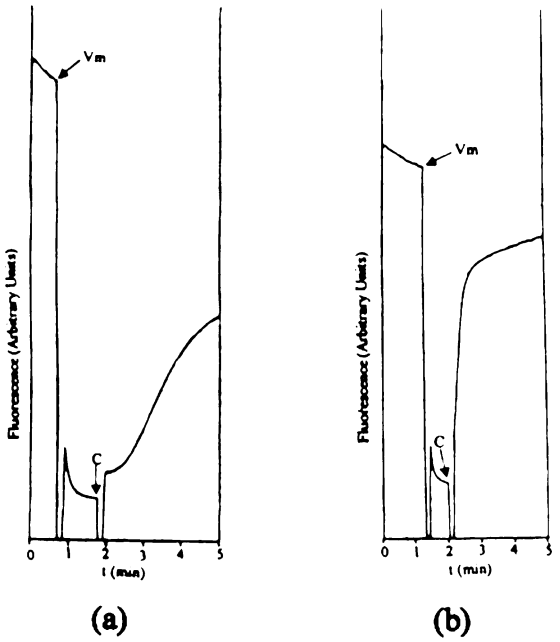

Figure 1. The profiles (a) and (b) represent dissipation of diffusion potential set up by valinomycin (lipid:Vm ratio = 1000:1) in small unilamellar vesicles (SUVs) of palmitoyl oleoyl phosphatidyl choline (POPC) on addition of **4c** (7.91 μM) and **5c** (6.32 μM), respectively, as monitored by the fluorescence of cyanine dye dis-C_2 (5). SUVs were diluted into a buffer (5 mM HEPES, pH 7.4) solution of 150 mM NaCl. Vm and C, respectively, denote the points at which valinomycin and cyclobisamides were added. The increase in fluorescence on addition of cyclobisamides indicates transport of Na^+ ions from the suspension medium into the vesicles. The spikes seen on the profile at each point (Vm and C) result from the opening of the chamber door.

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6. *General experimental procedure for the preparation of cyclo-4-oxa-heptane-1,7-bisamides* **4a–c** *and* **5a–c**: A solution of **1** (0.5 mmol, freshly prepared from the precursor dicarboxylic acid by refluxing with 2 M excess of $SOCl_2$ for 3 h, followed by drying in vacuo) in dry CH_2Cl_2 (10 mL) was added dropwise over a period of 0.5 h to a well-stirred and ice-cooled solution of **2a–c** or **3a–c** (0.5 mmol) in dry CH_2Cl_2 (250 mL) containing NEt_3 (1 mmol) and the mixture stirred at room temperature for 12 h. The reaction mixture was washed sequentially with 20 mL each of ice-cold 2N H_2SO_4, H_2O, and 5% aqueous $NaHCO_3$ and the organic layer dried over anhydrous $MgSO_4$ and

evaporated in vacuo. The residue was purified on a short column of silica gel using a mixture of $CHCl_3$/MeOH as eluent to afford the title cyclobisamides. Compound **4a**: mp 190–192°C; IR (KBr) ν_{max} 3318, 2957, 2925, 2855, 1760, 1647, 1514 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 2.56 (t, 4H, -CH_2CO), 3.24 (d, $J=6.4$ Hz, 4H, Cyst $C^{\beta}H_2$s), 3.66 (m, 2H, -OCH_2), 3.76 (s, 6H, -CO_2CH_3), 3.92 (m, 2H, -OCH_2), 4.93 (m, 2H, Cyst C^{α}Hs), 6.79 (d, $J=8.0$ Hz, 2H, Cyst NHs); FAB MS m/z 395 ([M+H]$^+$, 100%). Anal. calcd for $C_{14}H_{22}N_2O_7S_2$: C, 42.63; H, 5.62; N, 7.10; S, 16.26. Found: C, 42.15; H, 6.02; N, 6.56; S, 15.99. Compound **4b**: mp 195–197°C; IR (KBr) ν_{max} 3394, 3300, 2953, 2381, 2337, 1856, 1753, 1543 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 0.92 (d, 12H, Leu CH_3s), 1.65 (m, 6H, Leu ($C^{\beta}H_2$s+C^{γ}Hs)), 2.56 (m, 4H, -CH_2CO), 3.13 (m, 2H, Cyst $C^{\beta}H_2$s), 3.29 (m, 2H, Cyst $C^{\beta}H_2$s), 3.63 (m, 2H, -OCH_2), 3.73 (s, 6H, -CO_2CH_3), 3.94 (m, 2H, -OCH_2), 4.52 (m, 2H, Leu C^{α}Hs), 4.79 (m, 2H, Cyst C^{α}Hs), 7.28 (d, $J=8.2$ Hz, 2H, Cyst NHs), 7.33 (d, $J=7.4$ Hz, 2H, Leu NHs); ES MS m/z 621 ([M+H]$^+$, 100%). Anal. calcd for $C_{26}H_{44}N_4O_9S_2$: C, 50.30; H, 7.14; N, 9.03; S, 10.33. Found: C, 50.15; H, 7.24; N, 8.59; S, 10.09. Compound **4c**: mp 173–175°C; IR (KBr) ν_{max} 3304, 3073, 2908, 2851, 1848, 1539 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.66–2.04 (m, 30H, Adm Hs), 2.52 (m, 4H, -CH_2CO), 3.06 (m, 2H, Cyst $C^{\beta}H_2$s), 3.27 (m, 2H, Cyst $C^{\beta}H_2$s), 3.55 (m, 2H, -OCH_2), 3.90 (m, 2H, -OCH_2), 3.60 (m, 2H, Cyst C^{α}Hs), 6.05 (s, 2H, Adm NHs), 6.96 (d, $J=8.4$ Hz, 2H, Cyst NHs); FAB MS m/z 633 ([M+H]$^+$, 25%), 655 ([M+Na$^+$], 100%). Anal. calcd for $C_{32}H_{48}N_4O_5S_2$: C, 60.73; H, 7.64; N, 8.85; S, 10.13. Found: C, 60.55; H, 7.82; N, 8.66; S, 9.98. Compound **5a**: mp syrup; IR (neat) ν_{max} 3366, 2941, 2913, 2858, 1734, 1671, 1528 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.66–2.13 (m, 14H, Adm Hs), 2.43 (m, 2H, -CH_2CO), 2.76 (m, 2H, -CH_2CO), 3.64–3.83 (s+m, 10H, -CO_2CH_3+-OCH_2), 4.32 (m, 2H, Ser $C^{\beta}H_2$s), 4.65 (m, 2H, Ser $C^{\beta}H_2$s), 4.93 (m, 2H, Ser C^{α}Hs), 7.45 (d, $J=7.3$ Hz, 2H, Ser NHs); ES MS m/z 553 ([M+H]$^+$, 100%). Anal. calcd $C_{26}H_{36}N_2O_{11}$: C, 56.51; H, 6.57; N, 5.07. Found: C, 56.72; H, 6.88; N, 4.90. Compound **5b**: mp 88–90°C; IR (KBr) ν_{max} 3350, 2957, 2869 (sh), 1736, 1654, 1528 cm^{-1}; ^1H NMR (500 MHz, DMSO-d_6) δ 0.83 (d, 6H, Leu CH_3s), 0.88 (d, 6H, Leu CH_3s), 1.49 (m, 2H, Leu C^{γ}Hs), 1.59 (m, 4H, Leu $C^{\beta}H_2$s), 1.64–2.05 (m, 14H, Adm Hs), 2.27 (m, 2H, -CH_2CO), 2.51 (m, 2H, -CH_2CO), 3.54 (m, 2H, -OCH_2), 3.60 (s, 6H, -CO_2CH_3), 3.67 (m, 2H, -OCH_2), 4.16 (m, 4H, Ser $C^{\beta}H_2$s), 4.29 (m, 2H, Leu C^{α}Hs), 4.67 (m, 2H, Ser C^{α}Hs), 7.98 (d, $J=7.8$ Hz, 2H, Ser NHs), 8.35 (d, $J=8.4$ Hz, 2H, Leu NHs); ES MS m/z 779 ([M+H]$^+$, 9%), 801 ([M+Na]$^+$, 100%). Anal. calcd $C_{38}H_{58}N_4O_{13}$: C, 58.60; H, 7.51; N, 7.19. Found: C, 58.22; H, 7.28; N, 6.92. Compound **5c**: mp 142–145°C; IR (KBr) ν_{max} 3323, 2909, 2853, 1734, 1652, 1522 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$) δ 1.63–2.10 (m, 44H, Adm Hs), 2.40 (m, 2H, -CH_2CO), 2.65 (m, 2H, -CH_2CO), 3.69 (m, 4H, -OCH_2), 4.21 (m, 2H, Ser $C^{\beta}H_2$s), 4.58 (m, 2H, Ser $C^{\beta}H_2$s), 4.65 (m, 2H, Ser C^{α}Hs), 6.15 (s, 2H, Adm NHs), 7.63 (d, $J=7.4$ Hz, 2H, Ser NHs); ES MS m/z 792 ([M+H]$^+$, 28%), 814 ([M+Na]$^+$, 100%). Anal. calcd $C_{44}H_{62}N_4O_9$: C, 66.81; H, 7.90; N, 7.08. Found: C, 67.02; H, 8.28; N, 6.82.

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