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Sugar Derived Oxazolone Pseudotetrapeptide as *γ*-Turn Inducer and Anion-Selective Transporter

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Keywords

sugar amino acid, oxazolone, pseudo-peptides, peptidomimetics, ion transport

Abstract

The C_3-tetrasubstituted furanoid sugar amino acid derived linear amino acid dipeptide **8** and tetrapeptide **9** on intra-molecular cyclization afforded oxazolone pseudo-peptides **1** and **2a**, respectively, with formation of oxazole ring at the *C*-terminus. Conformational study of **2a** showed seven membered intramolecular C=O···HN (II) hydrogen bonding leading to the γ-turn conformation which is absent in the precursor tetrapeptide **9** thus indicating the role of oxazolone ring in γ-turn formation in **2a**. This fact was supported by the molecular modeling studies. The oxazolone ring containing pseudo-tetrapeptide **2a** was found to be better ion transporter than pseudodi-

peptide**1**.The ion selectivity of **2a** indicated Cl^-anion-selective transport via the antiport mechanism.

Introduction

Tetrasubstituted *α*-amino acid (TAA) derived peptidomimetics offer well defined turn structures due to the presence of stereochemically stable quaternary carbon centre [1]. For example, Toniolo and co-workers reported cyclopropane based TAA derived peptides that showed α-turn conformation and forms 3_{10}-helice conformation in higher oligomers [2-3]. Tanaka and co-workers reported the synthesis of TAA derived peptides from L/D-dimethyl tartarate which showed 3_{10} helical conformation [4]. Konig and co-workers have reported tetrahydrofuran TAA and converted to three dipeptides that demonstrated β-turn type conformation [5]. Amongst these, the use of sugar derived TAA in pepdidomimetics is less explored. Fleet and co-workers incorporated spiropeptides as well as tri-/tetra-peptides in to the TAA at the anomeric position of mannaofructose [6-8]. Stick and co-workers have reported the synthesis of tetrasubstitiuted sugar furanoid amino acid (TSFAA) derived homologated di-, tri-, tetra- and penta-peptides from D-glucofuranose derivative of which the linear TSFAA peptide showed a well defined helical array that are stabilized by internal hydrogen bonds [9-11]. Our group has reported *trans*-vicinal D-glucofuranoroic-3,4-di-acid having TAA framework and incorporated it in to the *N*-terminal tetrapeptide. sequence (H-Phe-Trp-Lys-Thy-OH) to get glycopeptide which acts as a α-turn inducer [12]. Recently, our group has synthesized acyclic- and cyclic- fluorinated peptides from C-3 fluorinated D-glucofuranoid amino acid and demonstrated their selective anion transport activity [13-14]. In continuation of our interest in developing new sugar derived cyclic peptides [15], we synthesized TFSAA derived dipeptide **8**

and tetra-peptide **9** from 3-oxo-D-glucofuranose derivative and attempted intramolecular cyclization to get corresponding cyclic peptides I and II (Figure 1) for ion transport activity study. In this process, we obtained oxazolone pseudo-peptides **1** and **2a** (Figure 1) and not cyclic peptides I/II. The NMR studies of oxazolone ring containing pseudotetra-peptide **2a** indicated γ-turn conformation that was stabilized by the seven membered (II)NH···O=C intramolecular hydrogen bonding which was noticed to be absent in linear tetrapeptide **9**. The oxazolone ring containing pseudo-tetrapeptide **2a** demonstrated selective Cl^- transport activity while; the pseudodi-peptide **1** showed less and linear tetra peptide **9** did not exhibit any ion transport activity. To the best of our knowledge, this is the first report on the formation of oxazolone peptides from TSFAA that induces γ-turn and demonstrate ion transport activity.

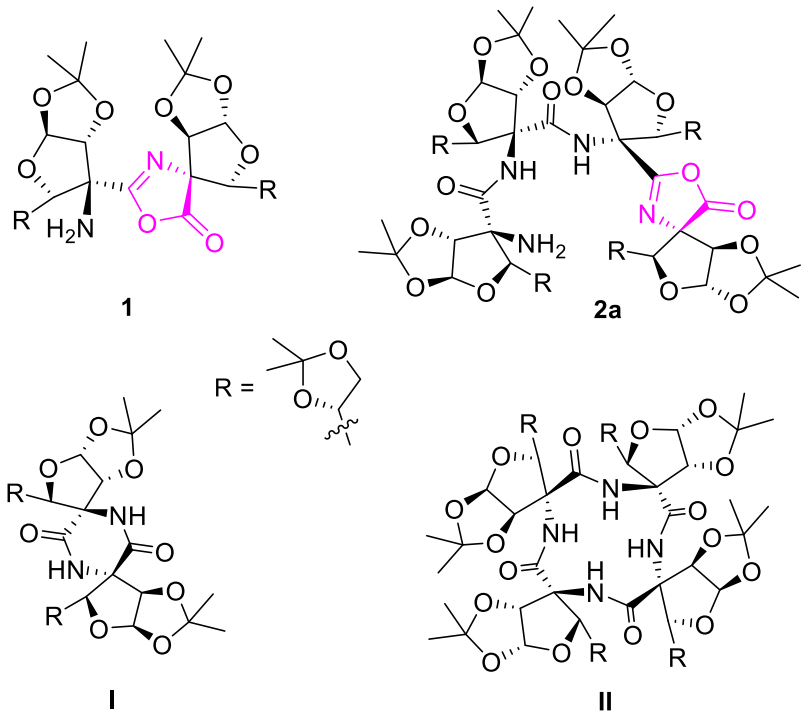
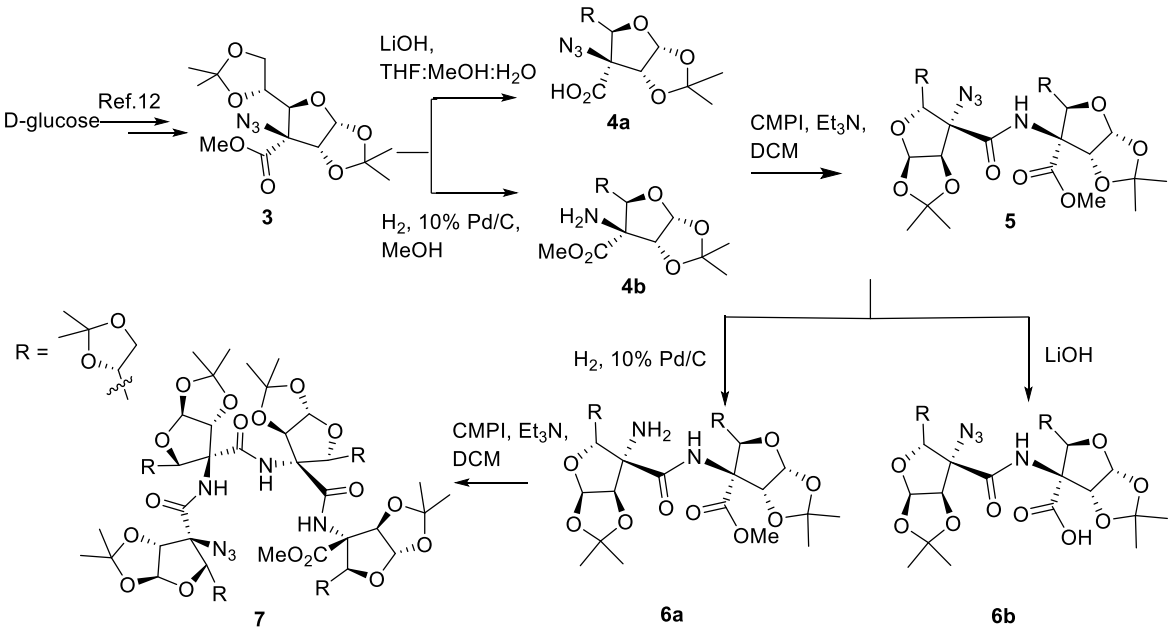


Figure 1: Pseudodi-peptide **1** and pseudotetra-peptide **2a** with oxazolone ring.

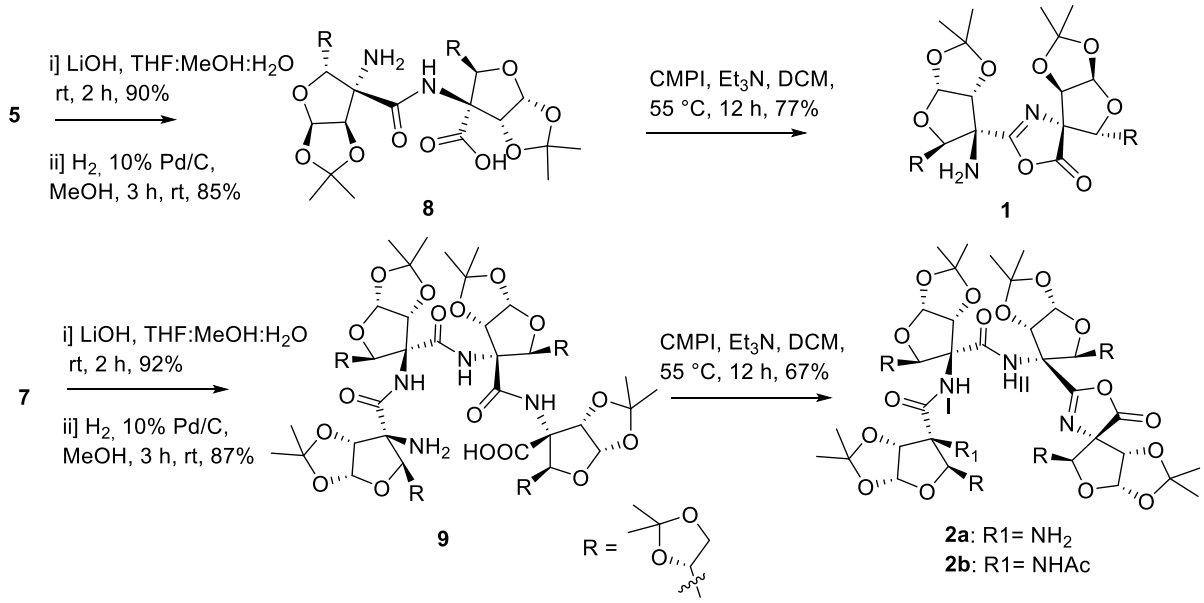
Results and Discussion

D-Glucose was converted to C_3-tetrasubstituted furanoid sugar azido ester **3** as per our reported protocol [12]. Hydrolysis of ester functionality in **3** using LiOH in THF:H_2O:MeOH at room temperture afforded azido acid **4a (**90%) while; hydrogenation of **3** using 10% Pd/C in MeOH at room temperture for 3 hours afforded amino ester **4b** in 85% yield. The coupling of **4a** and **4b** using CMPI as coupling reagent in the presence of Et_3N in dichloromethane at 55 ^oC for 12 hours gave azido ester dipeptide **5** in 75% yield. Hydrolysis of **5** using LiOH in THF:H_2O:MeOH gave azido acid dipeptide **6a** in 90% yield, while; hydrogenation of **5** using 10% Pd/C in methanol gave amino ester dipeptide **6b** in 87% yield.The coupling of **6a** and **6b** using CMPI ascoupling reagent in the presence of Et_3N in DCM afforded azido ester tetrapeptide **7** in 70% yield (Scheme 1) [16].



Scheme 1: Synthesis of azido ester peptide **5** and **7**.

The azido ester dipeptide **5** and tetrapeptide **7** were individually converted to amino acid di- and tetra-peptides **8** and **9** respectively, using hydrolysis followed by hydrogenation reaction protocol (Scheme 2).



Scheme 2: Synthesis of oxazolone pseudopeptides **1**, **2a** and **2b**.

In order to get cyclic peptides I and II (Figure 1), an individual intramolecular coupling reaction of **8** as well as **9** was attempted. Thus, reactions of **8**/**9** with different coupling reagents such asHATU, TBTU, PyBOP, EDC.HCl under different solvent (DMF, acetonitrile, dichloromethane) and reaction conditions (25-80 ^{o}C for 24 h) were unsuccessful. This could be due to stable helical conformation of **8** and **9** inwhichreactive acid and amino functionalities are apart from each other. However, individual intramolecular coupling reaction of **8** and **9** usingCMPI as a coupling reagent in the presence of Et_3N in dichloromethane afforded oxazolone pseudodi-peptide**1**and pseudotetra-peptide **2a**, respectively, with oxazolone ring formation at the *C*-terminal of the peptides [17-19]. The free amino group in **2a** was acetylated with Ac_2O, pyridine in DCM to get –NHAc derivative **2b**. The single crystal formation of oxazolone psudopeptides **1**, **2a** and **2b** was unsuccessful under variety of solvent conditions.

The ^{1}H and ^{13}C-NMR spectra of **1**, **2a** and **2b** showed sharp and well resolved signals in $CDCl_3$ solution indicating the absence of rotational isomers (Figure S9, S11, S12 in SI). The oxazolone pseudo-dipeptide **1** is devoid of amide linkages and

is therefore not considered for conformational studies [20]. In case of **2a**, the assignment of chemical shifts to different protons was made on the basis of ^{1}H-^{1}H COSY, ^{1}H-^{13}C-HMBC/HSQC, NOESY and ^{1}H-^{15}N HSQC/HMBC studies (S14-S18 in SI) and values thus obtained are given in Table S1. The IR spectrum of **2a** showed broad band at 3444 – 3421 cm^{-1} indicating the presence of amino as well as amide NHs. The bands at 1740 and 1688 cm^{-1} were assigned to the lactone carbonyl and amide (as well as imine) groups, respectively. In the ^{1}H NMR spectrum, the downfield signals at δ 9.03 and 8.52 were assigned to the amide NH-I and NH-II, respectively. The signal at δ 1.80, integrating for two protons, was assigned to the presence of NH_2 functionality. In the ^{13}C spectrum, appearance of signals at δ 170.8, 170.6 and 166.7 were assigned to the lactone/amide carbonyl functionalities. The signal at δ 163.0 was assigned to the -C=N functionality. The ^{1}H-^{15}N HSQC and ^{1}H-^{15}N HMBC spectra showed signal at δ 246.0 that was assigned to the imine (C=N-) nitrogen. The signal at δ 26.2 was assigned to the amine (NH_2) nitrogen. The signals at δ 112.8 and δ 114.1 were assigned to the nitrogen attached to the amide carbonyl (CONH).groups. This observed chemical shifts in the ^{15}N NMR were suggestive for the formation of oxazolone ring [17-19] at the *C*-terminus supporting structure **2a**.

The ^{1}H NMR spectra of *N*-acylated compound **2b** showed three downfield signals at δ 8.24, 8.19 and 8.09 that were assigned to three amide NHs. An additional singlet at δ 2.0, integrating for three protons, was assigned to the $NHCOCH_3$. In the ^{13}C NMR spectrum, appearance of five signals in the downfield region at δ 171.6, 170.9,167.5,165.0, and 164.0 indicated the presence of three amide, lactone carbonyl and imine carbon (-C=N) suggesting the presence of oxazolone ring in **2b**.

Conformational Study of 2a

The downfield shift of amide NH protons >δ 7.5 in **2a** suggested the possible involvement of intra-molecular hydrogen bonding [21]. The observed NOESY cross peaks of NH(I)↔NH_2 indicated closer proximity and are oriented on the same side (Figure 2). This is likely to involve (I)NH···NH_2 weak intramolecular hydrogen bonding. The amide NH(II) showed strong cross peaks with H-2, H-5 of ring C and H-4 of ring B and weak cross peaks with H-1, H-6 proton of ring C, indicating closer proximity and orientation on the same side of these protons. Appearance of strong NOE between NH(II)↔H-4 and weak NOE between NH(II)↔H-2 protons of ring B indicated that the NH(II) is bending towards the ring A carbonyl with the formation of seven membered ring intramolecular hydrogen bonding leading to the γ-turn conformation (Figure 2).

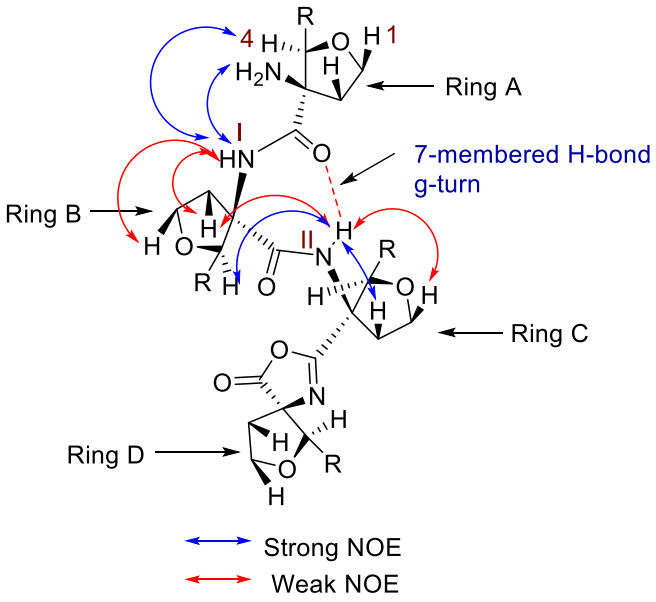


Figure 2: NOESY spectrum of **2a** andcharacteristic NOE.

The involvement of amide NHs in intramolecular H-bonding was supported by the DMSO-d_6 titration studies. Thus, 5 μL of DMSO- d_6 was sequentially added (up to 50 μL) to the $CDCl_3$ solution of **2a** and change in *δ* value of NH protons was monitored by the ^{1}H NMR [22]. The NH(I) proton showed higher change in chemical shift $\Delta\delta$ =

0.2 ppm indicating weak (I)NH···NH_2 intramolecular H-bonding, and NH(II) showedsmaller $\Delta\delta$ = 0.13 ppm suggesting strong (II)NH···O=C H-bonding (Figure 3).

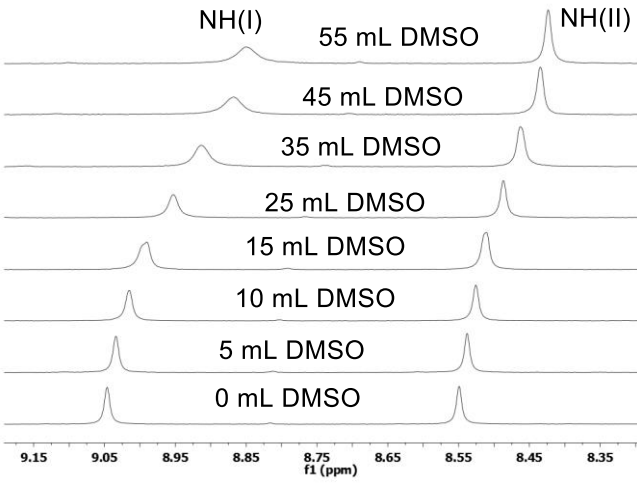


Figure 3: DMSO titration study of **2a.**

This fact was further supported by temperature dependent ^{1}H-NMR study [23-27]. The temperature dependent ^{1}H NMR of **2a** in $CDCl_3$ solvent at 283 – 323 °K was recorded that showed higher Δδ/ΔT value of 6.2 × 10^{-3} ppm/K for NH(I) indicating its involvement in weak intramolecular H-bonding. For NH(II) the lower Δδ/ΔT value of 3.7 × 10^{-3} ppm/K supported its association in strong intra-molecular hydrogen bonding with C=O leading to the *γ*-turn conformation (Figure 4).

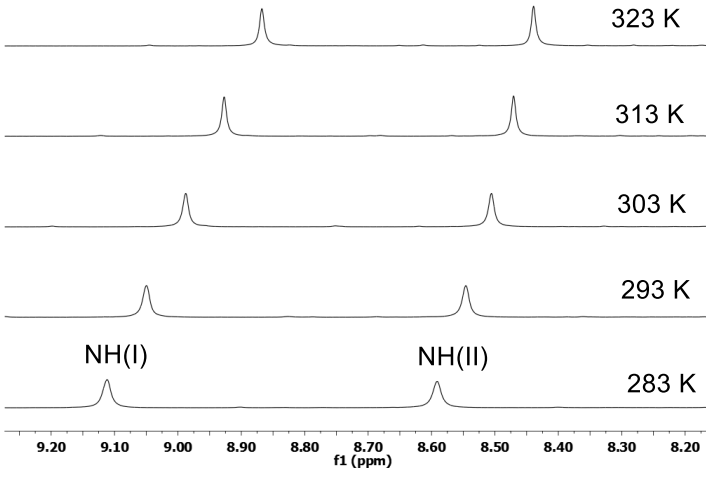


Figure 4: ^{1}H NMR temperature study of **2a.**

The ^{1}H NMR dilution study of **2a** in $CDCl_3$ solution showed negligible change ($\Delta\delta$ = 0.01) in the chemical shift of NH(I) and(II) protons (Figure S17), further supporting their intramolecular hydrogen bonding with the free NH_2 and C=O, respectively. These studies thus supported the presence of *γ*-turn helical type conformation of **2a**.

Molecular modeling studies

In order to corroborate our results obtained from the NMR studies, the molecular modeling study was performed using*Spartan'14* software [28-29]. The initial geometry of **2a,** generated from the NOESY study, was subjected for geometry optimization using semi-empirical PM6 method. The resulted optimized structure of **2a** indicated considerable crowding due to the presence of oxazolone ring and two acetonide rings of the sugar ring D (Figure 5A). To accumulate the oxozolone ring, the sugar ring C is pushed towards sugar rings B and A forming γ-turn conformation that is stabilized by seven membered intramolecular (II) NH···O=C hydrogen bonding (bond distance (*d*) = 2.61 Å and bond angle (NH···O) = 114.06°). To understand the role of oxazolone ring in stabilizing the γ-turn, we performed geometry optimization on TFSAA linear tetrapeptide amino acid **9 (**Figure 5C). The optimized geometry of **9** showed change in helical conformation to release the crowding due to acetonide groups wherein; the *N*- and C-terminals are further away- thus precluding the γ-turn conformation (bond distance (*d*) = 3.11 Å), and bond angle (NH···O) = 98.90°. The comparison of geometrically optimized models of **2a** and **9** showed small structural changes with respect to helical pitch length. The distance between C=O···N(II) is 3.18 Å in **2a** and 3.43 Å in **9.** The distance between $C\alpha_1 \cdots C\alpha_4$ is 9.67 Å in **2a** and9.84 Å in **9** (Figure S27 in SI). Similarly, distance between $N_1 \cdots C_4$ is 9.44 Å in **2a** and 10.47 Å in **9.** This suggested elongated helical structure of linear tetrapeptide**9** than **2a** thus supporting the compact helical architecture for **2a** due to the presence of oxozolone

ring leading to γ-turn conformation. The molecular modelling study of *N*-acetylated compound **2b** also indicated the presence of seven membered hydrogen bonding between NH(II) and –C=O ((bond distance (*d*) = 2.74 Å). and bond angle (NH···O) = 112.98°) suggesting the presence of γ-turn conformation (Figure 5B) due to the presence of oxozolone ring.

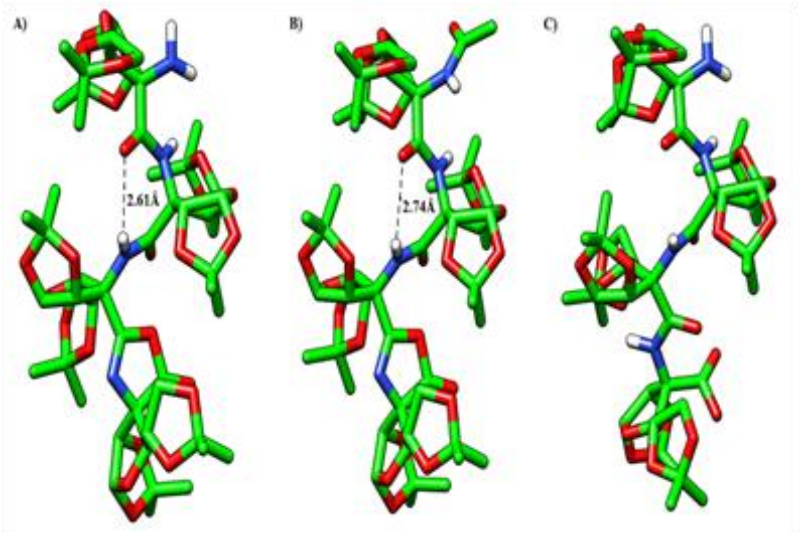


Figure 5: Optimized helical conformations of (A) **2a**, (B) **2b** and (C) **9**.

Ion transport activity

The cation and anion transport across lipid bilayer membrane plays a crucial role in various biological processes [30-32]. Amongst these, the transport of anions is useful in regulating intracellular pH, membrane potential, cell volume, and fluid transport [33]. Anydysfunction in these processes led to various diseases such as cystic fibrosis, Dent disease, Bartter syndrome, and epilepsy [34-41]. In order to mimic the regulatory functions in living systems, a wide range of anion transporters have been investigated that include peptides [42-51], oligoureas [52-53], anion-π slides [54-57],steroids [58-61], calixpyrroles [62-64],calixarenes [65-67], and other scaffolds [68-70]. In particular, peptide based transmembrane anion transporters have attracted

great interest. For example, Ghadiri [42], Ranganathan [43], and Granja [44] have independently reported different types of cyclic peptides as anion transporters. Gale, Luis and coworkers [45-46] have separately reported the linear pseudopeptides as a receptors and transporters of chloride and nitrate anions.

Inspired with our recent ion transport studies with acyclic and cyclic fluorinated sugar derived peptides [13-14], we investigated the ion transport activity of **1** and **2a** across lipid bilayer membrane. In this study, the collapse of the pH gradient (pH_{out} = 7.8 and pH_{in} = 7.0), created across egg yolk L-α-phosphatidylcholine (EYPC) vesicles with entrapped 8-hydroxypyrene-1,3,6-trisulfonic acid trisodium salt (HPTS) dye (i.e., EYPC-LUVs⊃HPTS) [71-78] was monitored by measuring the fluorescence intensity of the dye at λ_{em} = 510 nm (λ_{ex} = 450 nm) with time (Figure S11). Thus, addition of **2a** (10 μM) resulted in the significant increase in HPTS fluorescence within 200 s (Figure 6B), while oxazolone pseudodi-peptide **1** was found to be lesser active (Figure 6A).

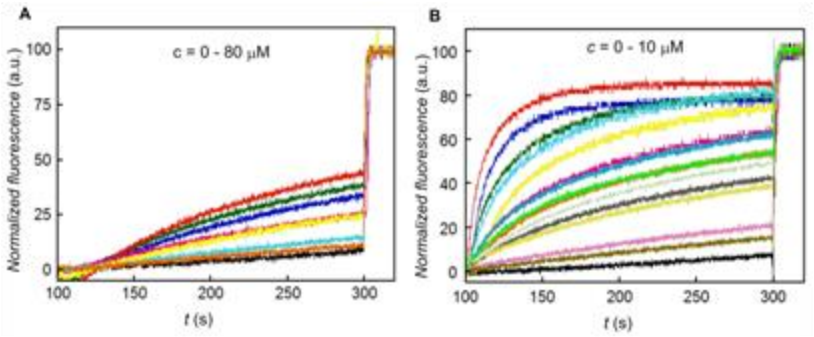


Figure 6: Ion transport activity (A) for **1**, (B) for **2a**, across EYPC-LUVs HPTS.

From the dose response data of **2a**, the calculated effective concentration EC_{50} = 0.72 μM indicated good ion transport activity of **2a** (Figure S12 in SI).The Hill coefficient *n* value of 1.26 indicated that one molecule of **2a** is involved in the formation of the active transporter. The promising ion transport activity of **2a** encouraged us to explore its cation and anion selectivity study by varying either cations (for MCl, M^+ = Li^+, Na^+, K^+, Rb^+, and Cs^+) or anions (for NaA, A^-,F^-, Cl^-, Br^-, I^-, NO_3^-, SCN^-, AcO^- and ClO_4^-) of the extra-vesicular salt, respectively. Thus,

variation of external cations, in the presence of **2a** (0-10 μM), showed minor changes in the transport activity with the sequence: $Na^+ > Rb^+ > Li^+ > K^+ \sim Cs^+$ (Figure 7A), which suggest lesser influence of alkali metal cations in the transport process. However, variation of extra-vesicular anions demonstrated the changes in the transport behaviour with the following selectivity sequence: $Cl^- >> AcO^- \sim SCN^- \sim F^- > NO_3^- >> Br^- \sim I^-$, showing highest selectivity for the Cl^- ion (Figure 7B). This data indicated the presence of $Cl^-\cdots HNH\cdots Cl^-$ interactions with anion. The absence of change in chemical shift of amide NH's is probably due to their involvement in strong intramolecular hydrogen bonding.

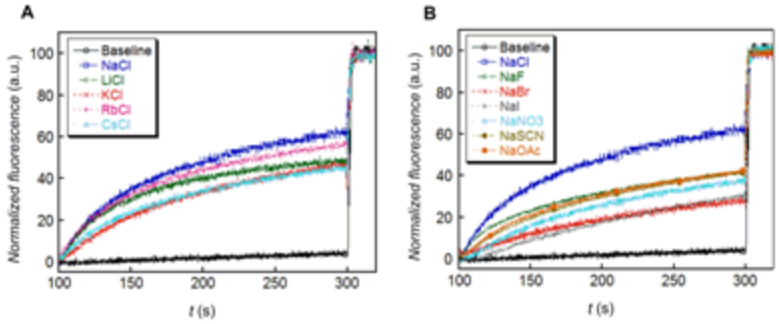


Figure 7: (A) Cation and (B) Anion transport activity of **2a**.

Chloride leakage study

In order to know the role of free NH_2 group in **2a** for Cl^- recognition during the transport of the ion, we monitored the Cl^- transport activities of the amino compound **2a** and its *N*-acylated derivative **2b**. The influx of Cl^- ion by these transporters were monitored using EYPC-LUVs⊃Lucigenin. Additionally, compound **9**, that has a free amino and a free carboxylic acid groups, was also subjected to the Cl^- transport study.The Lucigenin, a Cl^- sensitive dye, was entrapped within the lipid vesicles and the rate of quenching in fluorescence at λ_{em} = 535 nm (λ_{ex} = 455 nm) was monitored using transporter **2a** by creating a Cl^- gradient across the lipid membrane by applying NaCl in the extravesicular buffer (Figure S14 in SI). The compound **2a** showed a

significant decrease in the fluorescence rate of lucigenin and the change in fluorescence upon the addition of **2a** (Figure 8A and 8B). We observed that the *N*-acetylated compound **2b** to be inactive (Figure 8A) indicating that the free amine group is necessary for the transport activity. The compound **9** did not exhibit any transport activity even at very high concentration (Figure S16 in SI).

Further, the variation of cations in the extravesicular buffer using different salts of MCl (M^+ = Li^+, Na^+, K^+, Rb^+, Cs^+) does not make any change in the transport rate of **2a** (20 μM) which excludes any role of cation in an overall transport process (Figure 8C). Finally, to evaluate the mechanism of ion transport, the transport of Cl^- using the compound **2a** (20 μM) was monitored in presence and absence of valinomycin (a selective K^+ transporter, 1 μM). There was a significant increase in the transport rate of **2a** in presence of valinomycin confirming the transport process occurring through antiport mechanism via Cl^-/NO_3^- exchange (Figure 8D). This study suggest that the fact that the presence of oxazolone ring and free amino group are responsible for the ion transport and selective Cl^- activity of **2a**.

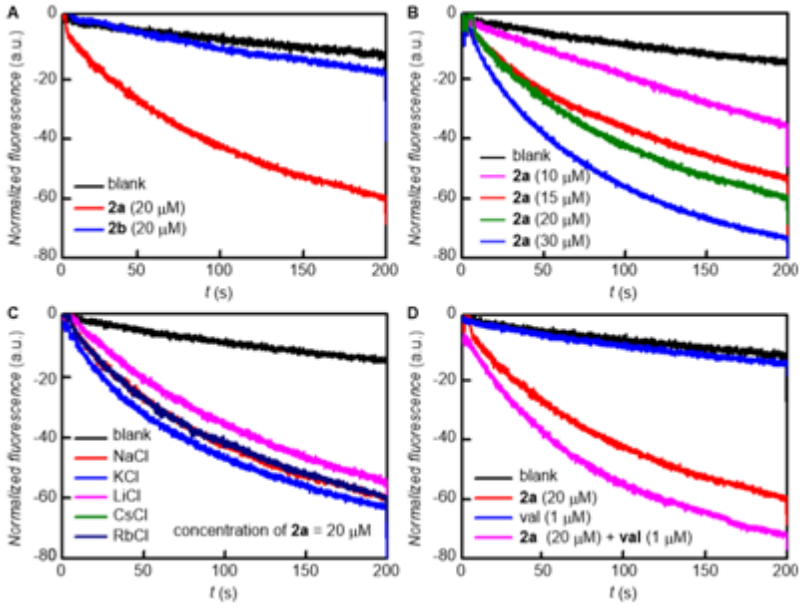


Figure 8: (A) Comparison of Ion Transport activity of **2a** and **2b** at 20 μM across EYPC-LUVs⊃Lucigenin (B) Concentration dependent activity of **2a** across EYPC-

LUVs⊃Lucigenin (C)Transport activity of **2a** (20 μM) by changing extravesicular cations (D) Transport activity of **2a** (20 μM) in the presence and absence of valinomycin (1 μM) across EYPC-LUVs⊃Lucigenin binding.

Conclusion

In conclusion, we have synthesized C3-TFSAA dipeptide **8** and tetrapeptide **9**. The intramolecular cyclization of **8** and **9** led to the formation of oxazolone ring at the C-terminal giving pseudo-peptides **1** and **2a**. The pseudo-tetrapeptide **2a** showed γ-turn conformation that is stabilized by the seven membered intramolecular hydrogen bonding. The pseudo-tetrapeptide **2a** was found to be selective ion transporter towards anions than alkali cations. The anion transport by **2a** was facilitated by an anion-anion antiport mechanism. The absence of γ-turn conformation as well as ion transport activity in linear tetra peptide **9** – the precursor of **2a**, suggest that the oxazolon ring in **2a** is γ-turn inducer as well as responsive for selective anion transport activity.

Supporting Information

Supporting information (Experimental procedures, mp, ^{1}H and ^{13}C NMR data, HRMS and 2D NMR) for this article is provided in supporting information

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References

1. Maity, P.; Konig, B.; *Pept. Sci*., **2008**, *90*, 8-27.
2. Bonora, G. M.; Toniolo, C.; Di Blasio, B.; Pavone, V.; Pedone, C.; Benedetti, E.; Lingham,I.; Hardy, P.; *J. Am. Chem. Soc*., **1984**, *106*, 8152-8156.
3. Toniolo, C.; Bonora, G. M.; Barone, V.;Bavoso, A.; Benedetti, E.; Di Blasio, B.;Grimaldi, P.;Lelj, F.;Pavone, V.; Pedone, C.; *Macromolecules*,**1985**, *18*, 895-902.
4. Demizu, Y.; Doi, M.; Kurihara, M.; Maruyama, T.; Suemune,H.; Tanaka, M.;*Chem. Eur. J.*, **2012**, *18*, 2430-2439.
5. Maity, P.; Zabel M.; Konig, B.; *J. Org.Chem.,* **2007***, 72,* 8046-8053.
6. Estevez, J. C.; Fstevez, R. J.; Ardron, H.; Wormald, M. R.;*Brown, D.;* Fleet, G.W. J.*; Tetrahedron Lett.,* **1994***, 35,* 8885-8888.
7. Estevez, J. C.; Fstevez, R. J.; Ardron, H.; Wormald. M. R.; Brown D.; Fleet,G.W. J.; *Tetrahedron Lett., **1994,** 35,* 8889-8890.
8. Estevez, J. C.; Smith, M. D.; Wormald, M. R.; Besra, G. S.; Brenna, P. J.; Nash, R. J.; Fleet, G. W. J.; *Tetrahedron: Asymmetry.,* **1996**, *7*, 391-394.
9. Forman, G. S.; Scaffidi, A.; Stick, R. V.; *Aust. J. Chem.,* **2004**, *57*, 25-28.
10. Scaffidi, A.; Skelton, B. W.; Stick, R. V.; White,A. H.; *Aust. J. Chem.,* **2007***, 60,* 93-94.
11. Scaffidi, A.; Skelton, B. W.; Stick, R. V.; white, A. H.; *Aust. J. Chem.,* **2004***, 57,* 733-740.
12. Vangala, M.; Dhokale, S. A; Gawade, R. L; Rajamohanan, R. P.; Puranik,V. G.; Dhavale, D. D.; *Org. Biomol. Chem*., **2013**, *11*, 6874-6878.

13. Burade, S. S; Shinde, S. V;Bhuma, N.; Kumbhar, N.; Kotmale, A.; Rajamohanan, P. R.; Gonnade, R. G; Talukdar, P.; Dhavale, D. D.; *J. Org. Chem.,* **2017**, *82*, 5826-5834.
14. Burade, S. S; Shinde, S. V; Bhuma, N.; Kumbhar, N.; Kotmale, A.; Rajamohanan, P. R.; Gonnade, R. G; Talukdar, P.; Dhavale, D. D.; *Org. Lett.,* **2017**,*19*, 5948-5951.
15. Pawar, N. J.; Diederichsen, U.; Dhavale, D. D.; *Org. Biomol. Chem*., **2015**, *13*, 11278-11285.
16. Synthesis of azido dipeptide **5** and tetrapeptide **7** is reported [11] using TsCl in pyridine as activating agent for carboxylic group. The same reaction at our hand gave dark brown colour product that on purification afforded ~30% yield while; the use of CMPI as coupling reagent gavepale yellowsolid product that on purification gave ~75% yield of **5** and**7**.
17. King, S. W.; Stammer, C. H.; *J. Org. Chem.,* **1981***, 46,* 4780-4782.
18. Yogisawa, S.;. Urakami. M; *Tetrahedron Lett.,* **1996***, 37,* 7557-7560.
19. Sakamoto, S.; Kazumi, N.; Kobayashi, Y.; Tsukano, C.; Takemoto. Y.; *Org. Lett.,* **2014***, 16, 4758-4761.*
20. Further, reactions of **1** and **2** under different acidic/basic conditions gave complex mixture of products thus precluding extension of work.
21. Nowick, J. S.; Smith,E. M.; Pairish, M.; *Chem. Soc. Rev.,* **1996**, *25,* 401-415.
22. El-Faham, A.; Albericio, F.; *Chem. Rev*., **2011**, *111*, 6557-6603.
23. Cung, M. T.; Marraud, M.; Neel, J.; Aubry, A.; *Biopolymers*, **1978**, *17*, 1149-1173.
24. Stevens, E. S; Sugawara, N.; Bonora; G. M.; Toniolo, C.; *J. Am. Chem. Soc.,* **1980**, *102*, 7048-7050.
25. Kessler, H. ; *Angew. Chem. Int. Ed.,* **1982**, *21*, 512-523.
26. Kishore, R.; Kumar, A.; Balaram, P.; *J. Am. Chem. Soc.,* **1985**, *107*, 8019-8023.

27. Gellman, S. H.; Dado, G. P.; Liang, G.-B;. Adams, B. R.; *J. Am. Chem. Soc.,* **1991**, *113*, 1164-1173.
28. Hehre, W. J.; Radom, L.;. Schleyer, P.V.R.; Pople, J. A.; *ab initio Molecular Orbital Theory,* Wiley, NY, **1986**.
29. J Stewart, J. J. P.; *J. Mol. Model.*, **2007**, *13*, 1173-1213.
30. Hille, B.; Ion Channels of Excitable Membranes, 3rd ed.; Sinauer Sunderland, MA **2001**.
31. Duran, C.; Thompson, C. H.; Xiao Q.; Hartzell, H. C; *Annu. Rev. Physiol*., **2010**, *72*, 95-121.
32. Benz, R.; Hancock, R. E. W; *J. Gen. Physiol.,* **1987**, *89*, 275-295.
33. Beer, P. D.; Gale, P. A.; *Angew. Chem. Int. Ed*., **2001**, *40*, 486-516.
34. Chloride movements across cellular membranes. In Advances inMolecular and Cell Biology; M. Pusch, Ed.; Elsevier: San Diego, **2007**; Vol *38*.
35. Jentsch, J. J. T; Stein, V.; Weinrich, F.; Zdebik, A. A.; *Phys. Rev.,* **2002**, *82*, 503-568.
36. Planells-Cases, R.; Jentsch, T. J.; *Biochim. Biophys. Acta Mol. Basis Dis.,* **2009**, *1792*, 173-189.
37. Cordat, E. J., Casey, R.; *Biochem. J.,* **2009**, *417*, 423-439.
38. F. M. Ashcroft, *Ion Channels and Disease: Channelopathies*, *Academic Press*, **2000**
39. Quinton, P.M.; *Lancet* **2008**, *372*, 415-417.
40. Busschaert N.; Gale, P. A.; *Angew. Chem. Int. Ed.,* **2013**, *52***,** 1374-1382.
41. Choi, J. Y.; Muallem, D.; Kiselyov, K; Lee, M. G.; Thomas, P. J.; Muallem, S.; *Nature,* **2001**, *410(6824)*, 94-97.
42. Bong, D. T.; Clark, T. D.; Granja, J. R.; Ghadiri, M. R.; *Angew. Chem., Int. Ed*., **2001**, *40*, 988-1011.

43. Ranganathan, D.; *Acc. Chem. Res*., **2001**, *34*, 919-930.
44. Brea, R. J.; Reiriz, C.; Granja, J. R.; *Chem. Soc. Rev*., **2010**, *39*, 1448-1456.
45. Martí, I.; Burguete, M. I.; Gale, P. A.; Luis, S. V.; *Eur. J. Org. Chem.,* **2015**, 5158.
46. Mart, I.; Bolte, M.; Burguete, M. I.; Vicent, C.; Alfonso,I; Luis, S. V.; *Chem. Eur. J.,* **2014**, *20*, 7458-7464.
47. Robert, B. P.; Elmes and Katrina; Jolliffe, A.; *Chem. Commun.,* **2015**, *51*, 4951-4968.
48. Shank, L. P.; Broughman, J. R.; Takeguchi, W.; Cook, G.; Robbins, A. S.; Hahn, L.; Radke, G.; Iwamoto, T.; Schultz, B. D.; Tomich, J. M.; Biophys. J., **2006**, *90*, 2138-2150.
49. Schlesinger, P. H.; Ferdani, R.; Liu, J.; Pajewska, J.; Pajewski, R.; Saito, M.; Shabany ,H.; Gokel, G. W.; *J. Am. Chem. Soc*., **2002**, *124*, 1848-1849.
50. Benke, B. P.; Madhavan, N.; *Chem. Commun.,* **2013**, *49*, 7340-7342.
51. Benke, B. P.; Madhavan , N.; *Bioorg. Med. Chem*., **2015**, *23*, 1413-1420.
52. Dieme, V.; Fischer, L.; Kauffmann, B.; Guichard, G.; *Chem. Eur. J.,* **2016**, *22*, 15684-15692.
53. Li, A.-F.; Wang, J.-H.; Wang, F.; Jiang, Y.-B.; *Chem. Soc. Rev.*, **2010**, *39*, 3729-3745.
54. Gorteau, V.; Bollot, G.; Mareda, J.; Perez-Velasco, A.; Matile, S.; *J. Am. Chem. Soc.,* **2006**, *128*, 14788-14789.
55. Gorteau, V. Bollot, G.; Mareda, J.; Matile, S.; *Org. Biomol*. *Chem.,* **2007**, *5*, 3000-3012.
56. Gorteau, V.; Julliard, M. D.; Matile, S.; *J.; Membr. Sci*., **2008**, *321*, 37-42.
57. Mareda, J.; Matile, S.; *Chem. Eur. J., **2009***, *15*, 28-37.
58. McNally, B. A.; Koulov, A. V.; Smith, B. D.; Joos, J.-B; Davis, A. P.; *Chem. Commun.,* **2005**, 1087-1089.

59. McNally, B. A.; Koulov, A. V.; Lambert, T. N.; Smith, B. D.; Joos, J.-B.; Sisson, A. L.; Clare, J. P.; Sgarlata, V.; Judd, L. W.; Magro, G.; Davis, A. P.; *Chem. Eur. J.* **2008**, *14*, 9599-9606.
60. Judd, L. W.; Davis, A. P.; *Chem. Commun*., **2010**, *46*, 2227-2229.
61. Hussain, S.; Brotherhood, P. R.; Judd, L. W.; Davis, A. P.; *J. Am. Chem. Soc.,* **2011**, *133*, 1614-1617.
62. Tong, C. C.; Quesada, R.; Sessler, J. L; Gale, P. A.; *Chem. Commun.,* **2008**, 6321-6323.
63. Fisher, M. G.; Gale, P. A.; Hiscock, J. R.; Hursthouse, M. B; Light, M. E.; Schmidtchen, F. P.; Tong. C. C.; *Chem. Commun.,* **2009**, 3017-3019.
64. Gale, P. A.; Tong, C. C.; Haynes, C. J. E.; Adeosun, O.; Gross, D. E.; Karnas, E.; Sedenberg, E. M.; Quesada, R.; Sessler, J. L.; *J. Am. Chem.Soc.,* **2010**, *132*, 3240-3241.
65. Sidorov, V.; Kotch, F. W.; Abdrakhmanova, G.; Mizani, R.; Fettinger, J. C.; Davis, J. T.; *J. Am. Chem. Soc.,* **2002**,*124*, 2267-2278.
66. Okunola, O. A.; Seganish, J. L.; Salimian, K. J.; Zavalij, P. Y.; Davis, J. T.;*Tetrahedron,* **2007**, *63*, 10743-10750.
67. Maulucci, N.; Izzo, I.; Licen, S.; Maulucci, N.; Autore, G.; Marzocco, S.; TecillaDe, P.; Riccardis, F.; *Chem. Commun.,* **2008**, 3927-3929.
68. Davis, J. T.; Okunola, O.; Quesada, R.; *Chem. Soc. Rev.,* **2010**, *39*,3843-3862.
69. Brotherhood, P. R.; Davis, A. P.; *Chem. Soc. Rev.,* **2010**, *39*, 3633-3647.
70. Gale, P. A.; *Acc. Chem. Res.,* **2011**, *44*, 216-226.
71. Kano,K.; Fendler, J. H.; *Biochim. Biophys. Acta. Biomembr*., **1978**, *509*, 289-299.
72. Clement, N. R.; Gould, J. M.; Biochemistry **1981**, 1534-1538.
73. Madhavan, N.; Robert , E. C.; Gin, M. S.; *Angew. Chem. Int. Ed.,* **2005***, 44,* 7584-7587*.*

74. Saha, T.; Dasari, S.; Tewari, D.; Prathap, A.; Sureshan, K. M.; Bera, A. K.; Mukherjee, A.; Talukdar, P.; *J. Am. Chem. Soc.,* **2014**, *136*, 14128-14135.
75. Kelly, V.T. R.; Kim, M. H.; *J. Am. Chem. Soc.,* **1994,** *116*, 7072-7080.
76. Dias, C.M.; Li, H.; Valkenier, H.; Karagiannidis, L. E.; Gale, P. A.; Sheppard; D. N. Davis, A. P., *Org. Biomol. Chem*., **2018**, *16*, 1083-1087.
77. Salunke, S. B.; Malla, J. A.; Talukdar P.; *Angew. Chem. Int. Ed*., **2019**, *58*, 1-6.
78. Hibberta D. B.; Thordarson, P.; *Chem. Commun*., **2016**, *52*, 12792-12805.