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Acyclic αγα-Tripeptides with Fluorinated- and Nonfluorinated-Furanoid Sugar Framework: Importance of Fluoro Substituent in Reverse-Turn Induced Self-Assembly and Transmembrane Ion-Transport Activity

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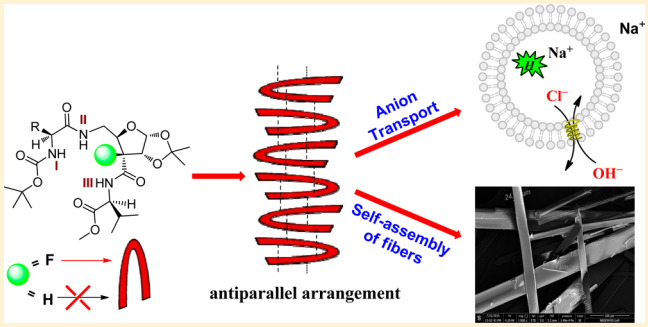
Supporting Information

ABSTRACT: Acyclic αγα-tripeptides derived from fluorinated-furanoid sugar amino acid frameworks act as reverse-turn inducers with a U-shaped conformation, whereas the corresponding nonfluorinated αγα-tripeptides show random peptide conformations. The NMR studies showed the presence of bifurcated weak intramolecular hydrogen bonding (F···HN) and $N^{+}\cdots F^{\delta-}$ charge-dipole attraction compel the amide carbonyl groups to orient antiperiplanar to the C−F bond, thus, demonstrating the role of the fluorine substituent in stabilizing the U-shaped conformation. The NOESY data indicate that the U-shaped tripeptides self-assembly formation is stabilized by the intermolecular hydrogen bonding between C═O···HN with antiparallel orientation. This fact is supported by ESI-MS data, which showed mass peaks up to the pentameric self-assembly, even in the gas phase. The morphological analysis by FE-SEM, on solid samples, showed arrangement of fibers into nanorods. The antiparallel self-assembled pore of the fluorinated tripeptides illustrates the selective ion-transport activity. The experimental findings were supported by DFT studies.



INTRODUCTION

The reverse-turn or loop structural motif is an intrinsic feature for adopting secondary structures in peptides.[1,2] These secondary structures, known as helices or β-sheets, extend to self-assembled tubular structures that are useful in biomedical aspects related to receptor recognition, substrate specificity, ion channel/transporter, and catalytic-function activity.[3] In general, the turn motifs are stabilized by intra/inter-residual interactions, conformational constraints, side-chain participation, and hydrophobic/hydrophilic interactions. Among a variety of peptidomimetics, those derived from sugar amino acids (SAA)[4] have attracted much interest because of their conformational ring constraints and easy access to get α/β/γ/δ-sugar amino acid frameworks for peptide bonding.[5] As a result, a number of SAA-derived peptides were investigated as reverse-turn mimetics. However, the fluorinated-furanoid sugar amino acid (FFSAA)- derived peptidomimetics have not been explored so far. The fluorine substitution within a molecule is known to alter its biophysical and chemical properties.[6] Particularly, the fluorine substituent in peptides stabilizes the secondary structures, self-assembled nanotubes[7], and also enhances the bioactivity.[8] For example, Seebach[9] and Abell[10] have independently reported the control of secondary structures of β-peptides by introduction of fluorine. O'Hagan and co-workers[11] synthesized a variety of fluorinated peptides in which peptide conformations are influenced by the stereoelectronic effect of fluorine. Hunter and co-workers[12] synthesized vicinal difluorinated analogue of cyclic heptapeptide *Unguisin A* that adopt different secondary structures controlled by the stereochemistry of fluorine. Aitken and co-workers[13] studied β-peptides of *cis*-2-amino-1-fluorocyclobutane carboxylic acid and demonstrated the strong conformational preference for a strand-like structure. As a part of our interest in SAA-derived peptides,[14] we recently incorporated sugar furanoid *trans*-vicinal diacid into the *N*-terminal tetrapeptide sequence (H-Phe-Trp-Lys-Thr-OH) to get glycopeptides that adopt a γ-turn conformation toward the *N*-terminal (Scheme 1A).[14a] Now, we report the synthesis of acyclic αγα-tripeptides derived from FFSAA, namely, BocNH-Ala-FFSAA-Val-OMe **9a**, BocNH-

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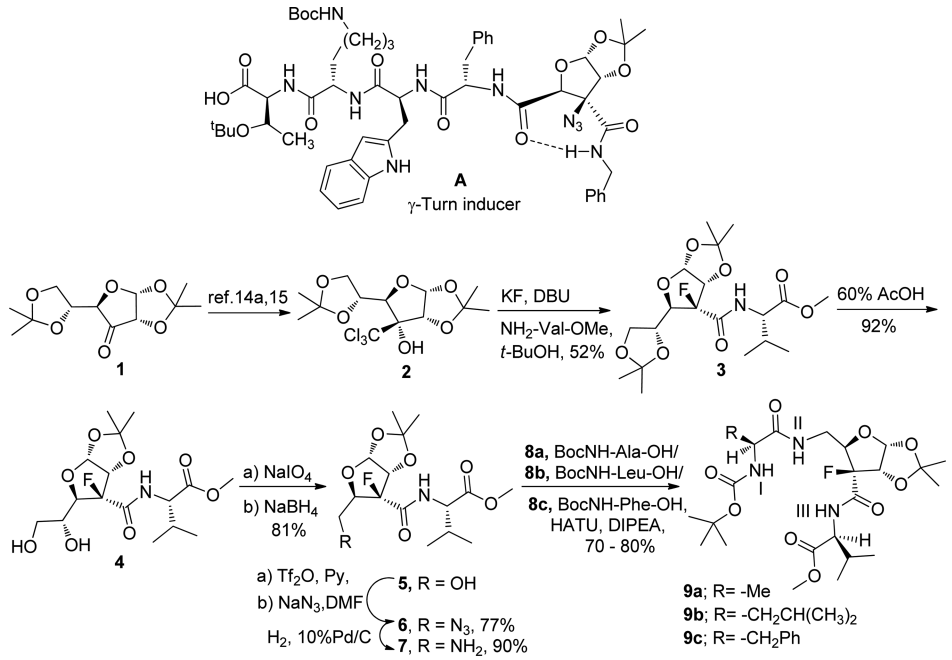
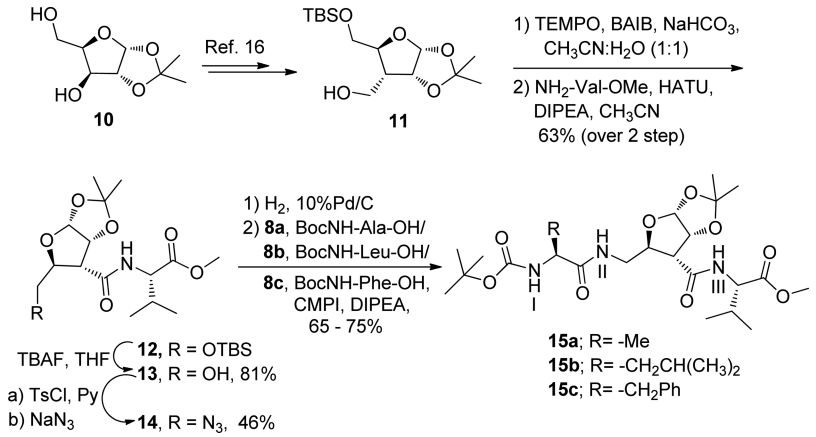
Scheme 1. Synthesis of Fluorinated $\alpha\gamma\alpha$-Tripeptides 9a, 9b, and 9c from D-Glucose



Scheme 2. Synthesis of Nonfluorinated $\alpha\gamma\alpha$-Tripeptides 15a, 15b, and 15c from D-Xylose



Leu-FFSAA-Val-OMe **9b**, BocNH-Phe-FFSAA-Val-OMe **9c**, and corresponding nonfluorinated SAA-derived acyclic tripeptides BocNH-Ala-SAA-Val-OMe **15a**, BocNH-Leu-SAA-Val-OMe **15b**, and BocNH-Phe-SAA-Val-OMe **15c.** The comparative study indicated that SAA-derived peptides **15a–c** showed random conformation, whereas the FFSAA-derived peptides **9a–c** act as reverse-turn inducers with the U-shaped conformation, suggesting the role of fluorine. The 2D-NMR and DFT studies of **9a–c** indicated weak intramolecular H-bonding (F···HN II and III) and alignment of C=O groups of amides antiperiplanar to the C–F bond inducing the reverse-turn conformations. These secondary structures aggregate into antiparallel self-assembled helical stacks, leading to the formation of nanorods (for **9a** and **9b**) and render the transmembrane ion transport activity. The formation of self-assembly is also evident from ESI-MS studies in solution and gas phase that showed mass peaks up to the pentameric self-assembly. The tripeptide **9c** did not show extended self-assembly because of the conformational constraints imposed by the phenyl ring. These results are supported by the DFT studies.

RESULTS AND DISCUSSION

Synthesis. 3-Oxo-α-D-glucofuranose **1** was converted to **2** as per reported protocol.[14a,15] Treatment of **2** with KF and HCl·H_2N-Val-OMe, using DBU in *t*-BuOH afforded 3-(*S*)-fluoro-Val-OMe **3** in 52% yield (Scheme 1). Deprotection of 5,6-acetonide functionality with 60% AcOH afforded a 5,6-diol **4** that on treatment with $NaIO_4$, followed by reaction with $NaBH_4$, afforded alcohol **5**. Treatment of **5** with Tf_2O in pyridine followed by reaction with NaN_3 in DMF afforded C-5-azido compound **6**. In the next step, hydrogenation of **6** using 10% Pd/C in methanol afforded C-5 amino compound **7** that on individual coupling with BocNH-Ala-OH **8a**, BocNH-Leu-OH **8b**, and BocNH-Phe-OH **8c** using HATU and DIPEA afforded linear $\alpha\gamma\alpha$-tripeptides **9a**, **9b**, and **9c**, respectively.

Our attempts to synthesize nonfluorinated analogues **15a–c** (analogous to **9a–c**) from intermediate **2**, under a variety of reaction conditions, were unsuccessful. Alternatively, the synthesis of **15a–c** was achieved from 3–hydroxymethyl–α–D–xylofuranose derivative[16a,b] **11** (Scheme 2). Thus, compound **11** treated with TEMPO, BAIB, and $NaHCO_3$ in

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Table 1. ^{1}H NMR Assignment of Amide NHs with Chemical Shift (δ ppm), Multiplicity, and Coupling Constant (J in Hz) for Tripeptides 9a–c and 15a–c

proton	9a	9b	9c	15a	15c	15b
NH(I)	5.11 (bs)	4.94 (d, J = 8.2)	5.09 (bs)	5.11 (d, J = 6.6)	4.94 (d,J = 6.6)	5.10 (d,J = 6.3)
NH(II)	6.40 (bs)	6.42 (bs)	6.24 (bs)	6.76 (bs)	6.75 (t, J = 5.6)	6.35 (bs)
NH(III)	6.92 (dd, J = 8.2, 2.6)	6.97 (bd, J = 8.3)	6.95 (dd, J = 8.3, 2.5)	7.18 (d, J = 8.3)	7.19 (d, J = 8.0)	6.98 (d,J = 8.3)

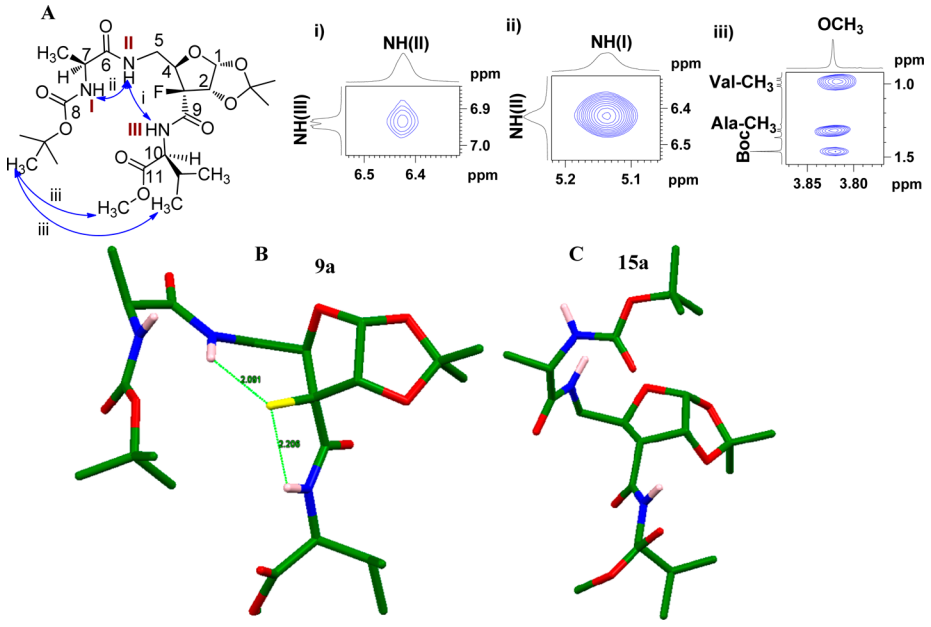

Figure 1. Characteristic intramolecular NOESY regions with schematic representation (blue arrow) of **9a** (A). Minimum energy structures of **9a** (B) and **15a** (C). The NH···F intramolecular H-bond interaction was calculated from DFT.

CH_3CN/H_2O afforded an acid derivative which was directly reacted with H_2N-Val-OMe in the presence of HATU and DIPEA to get **12.** Deprotection of the -OTBS group in **12** by TBAF gave alcohol **13,** which on reaction with TsCl in pyridine followed by NaN_3 in DMF afforded azide **14**. Hydrogenation of **14** using 10% Pd/C in methanol (to get a free amine), followed by individual coupling with amino acids **8a**, **8b**, and **8c** using CMPI and DIPEA, afforded linear, nonfluorinated SAA *αγα*-tripeptides **15a**, **15b**, and **15c**, respectively. Crystal formation of tripeptides **9a–c** and **15a–c** was attempted; however, only **9a** and **9b** resulted in needle-shaped crystals in ethyl acetate/hexane (1:1) that showed disorders in single-crystal X-ray diffraction studies.[16c]

Conformational Analysis. The ^{1}H NMR of **9a–c** and **15a–c** in $CDCl_3$ showed strongly resolved signals and the absence of rotational isomers (Figure S6–S8 and S12–S14). The chemical shifts and coupling-constant values for amide NHs for **9a–c** and **15a–c** are given in Table 1.

From Table 1, the $^4J_{H,F}$ = ∼ 2.5 Hz in **9a–c** suggested a *gauche* conformation between the C–F and N–H(III), thus making the C–F and C═O bond alignments antiparallel. Moreover, the indication of $^3J_{NH,\alpha H}$ > 8.0 Hz for NH(III) (in **9a–c** and **15a–c**) and NH(I) (only in **9b**) indicated a *trans* conformation.[17] The NOESY spectra of **9a–c** (Figure 1 and S15A) showed characteristic intramolecular NOEs between NH(II)↔NH(III), NH(I)↔NH(II), Boc–CH_3↔Val–CH_3, and Boc–CH_3↔OCH_3. This data indicated that the Cα'–N bond takes a reverse turn, leading to the U-shaped conformation. This fact is supported by the ^{1}H–^{19}F HOESY spectra of **9a–c** (Figure S22–S24) which showed through-space interactions between the fluorine atom, NH(II), NH(III), and the methylene protons at C-5. Although the NOESY of the nonfluorinated tripeptides **15a–c** (Figure S26, S28, and S30) showed the absence of NOESY cross-peaks between NH(II)↔NH(III), Boc–CH_3↔Val–CH_3 and Boc–CH_3↔OCH_3. Instead, we observed intramolecular NOE cross-peaks between the Boc–CH_3, and the H-1, H-2, and NH(I) protons of the sugar molecule, indicating that the two arms of tripeptides are oriented in the opposite direction. This gives the positive evidence of the nonfluorinated tripeptides adopting a nonturn structure **15a–c**.

In order to corroborate our experimental results obtained by NMR studies of fluorinated **9a–c** and nonfluorinated **15a–c** *αγα*-peptides, we have performed DFT calculations (see SI). The optimized conformations of tripeptides **9a–c** showed a reverse-turn U-shaped conformation, even in the absence of the much-preferred intramolecular N–H···O═C hydrogen-bonding interactions that are generally observed in peptides[1b,c] as shown in Figure 1B and S31. The reverse- turn U-shaped conformations in **9a–c** are stabilized by the fluorine substituent with the involvement of bifurcated weak intramolecular hydrogen bonding[6a] between F···HN (II) and F···HN (III). In addition, the adjacent amide N–H(III) adopts a *gauche* relation with the C–F because of the $N^{+}\cdots F^{\delta-}$ charge-dipole attraction, whereas the C═O groups take up an antiparallel orientation with respect to the C–F because of dipole repulsion. Thus, all the three amide N–H's are in *syn* orientation while -C═O groups are *anti* with respect to C–F bond. The corresponding nonfluorinated analogue **15a** showed random conformation, (Figure 1C) whereas **15b** showed 12-

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membered H-bonding (pseudohelical turn) between Boc−C═O···HN(III), compound **15c** showed 7-membered H-bonding (usually called as γ-turn) between Boc−C═O···HN(II) (Figure S31). This unexpected optimized structure of **15b** and **15c** suggested the substituent effect of the *N*-terminal α-amino acid (Leu and Phe) in DFT calculation. The calculated torsion angles and intramolecular H-bonding interactions are in agreement with reverse-turn conformation for **9a−c** (Table S3 and S4). This optimized DFT data of fluorinated and nonfluorinated $\alpha\gamma\alpha$-tripeptides **9a−c** and **15a−c** were in agreement with the NMR studies, suggesting the role of the fluorine substituent in fluorinated tripeptides **9a−c** in the stabilization of the reverse-turn U-shape conformations over the nonfluorinated tripeptides **15a-c.**

Studies on Self-Assembly Formation. The self-assembly structure formation in **9a** and **9b** is evident from the weak NOE's between NBoc−CH_3↔H-1, H-2 and H-4 of sugar ring and Ala−CH_3↔OCH_3 in **9a** and NBoc−CH_3↔H-1, H-2, and H-4 of the sugar ring in **9b.** This indicated the existence of intermolecular head-to-tail antiparallel self-association in **9a**/**9b** that is stabilized because of the possible intermolecular H-bonding between (I)NH···O═C (C-9) and (III)NH···O═C (C-6) (Figure 2 and S15B). Additional evidence for self-assembly is generated from the ESI mass spectrum of **9a** and **9b** that showed characteristic peaks due to $[M + Na]^+$ consistent with the mono-, di-, tri-, tetra-, and penta-meric aggregates (Figure S32 and S33), suggesting the formation of an extended self-assembled structure for fluorinated tripeptides **9a**, **b** in solution, as well as in the gas phase.

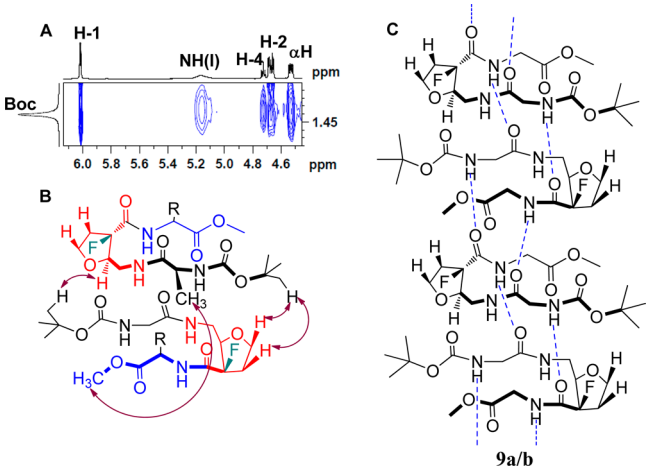

Figure 2. Selected NOESY region and schematic representation of intermolecular NOEs observed because of molecular self-association of **9a** (A and B) and model structure for arrangement of antiparallel self-assembly of **9a** and **9b**(C). Side chain functionalities are removed for sake of clarity.

The involvement of amide NH(I) and NH(III) in intermolecular H-bonding in self-assembly formation is evident from the temperature-dependent ^{1}H NMR study for compounds **9a**, **b** in $CDCl_3$ (Figure S36−S37).[18a,b] The chemical shift of NH(I) (~5.0 δ) in **9a**, **b** is indicative of the absence of the intramolecular H-bond.[19] However, the observed low value of the temperature coefficient ($\Delta\delta/\Delta T \approx 3.0$ ppb/K) is suggestive of involvement in intermolecular H-bonding (NH···O═C) in aggregate structure. Although the NH(II) and NH(III) are involved in the weak intramolecular NH···F bonding (δ 6.4 and 6.9 ppm), the NH(II) and NH(III) showed temperature coefficient values of ~3.1 and 1.0 ppb/K, respectively. The relatively low value (1.0 ppb/K) of $\Delta\delta/\Delta T$ in NH(III) is suggestive of its effective participation in intramolecular NH···F and intermolecular NH···O═C H-bonding in the self-assembled structure. This fact is further evident from the D_2O-exchange experiment of **9a** (Figure S38). The exchange rate after 4 days for NH(I) and NH(III) protons is ~20% as opposed to 60% for the NH(II) proton.[18c]

The CD analysis of **9a** and **9b** in methanol solution showed a negative band at ~224 nm and a positive band at ~197 nm, suggesting formation of an antiparallel self-assembly through intermolecular H-bonding (helical stacking). Tripeptide **9c** showed positive peaks at 198 and 221 nm with high and low molar ellipticity, respectively, indicating the absence of a helical superstructure (Figure S39).[20] However, nonfluorinated tripeptides **15a**, **b** showed a positive peak at ~207 nm and a negative peak at ~195 and ~227 nm, whereas **15c** showed positive peaks at 200 and 220 nm and a negative peak ~195 nm (Figure S39). Thus, the nonfluorinated tripeptides showed a shift of maxima and minima to a higher wavelength (as compared to fluorinated tripeptides) indicating the presence of a nonturn conformation.

SEM Analysis. As the X-ray crystal study of **9a** and **9b** showed many disorders and, thus, precluded its solid state structure, we performed morphological studies of tripeptides **9a−c** by scanning electron microscopy (Figure 3 and S40−S42). Tripeptides **9a** and **9b** form well-defined nanorod-like structures with 2 to 50 μm widths and several hundreds of microns length. However, **9c** showed the absence of a nanorod assembly.

Ion-Transport Activity. The self-assembly of **9a**, **b** into the antiparallel fibers (Figure 2C) allowed us to evaluate the ion transport through the pore upon placing the self-assembled structure in the phospholipid-bilayer membrane. The collapse

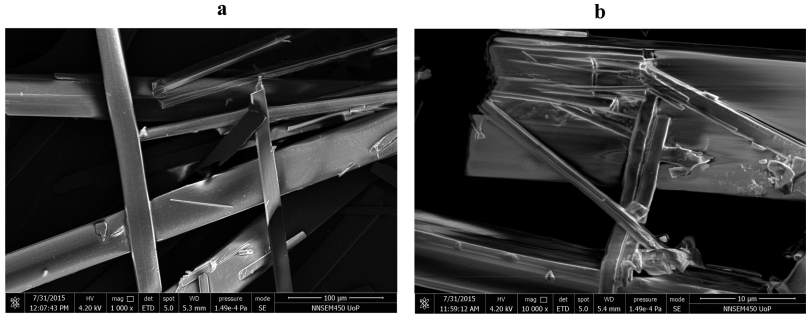

Figure 3. SEM images indicated nanorod formation in **9a** and **9b**.

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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,[21] was monitored by measuring the fluorescence intensity of the dye at λ_{em} = 510 nm (λ_{ex} = 450 nm) with time (see Figure S43). In this experiment, the ion-transport activity through the nanopore is indicated by the increase in the fluorescence intensity of the pH-sensitive dye. Interestingly, the addition of **9a–c** (20 μM) resulted in the significant increase in HPTS fluorescence, measured by an 18%, 60%, and 44% jump of fluorescence intensity within 200 s (Figure 4A). From the dose-response activity data of most

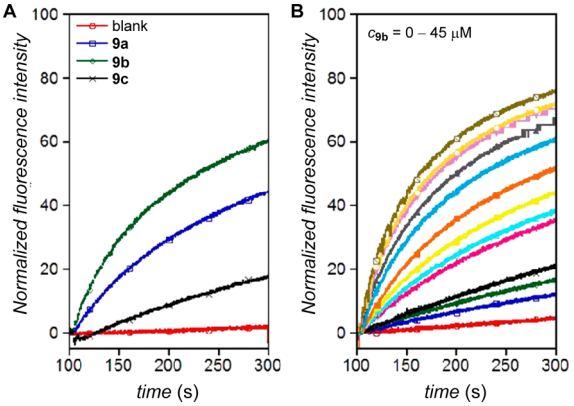

Figure 4. Comparison of ion-transport activities of acyclic αγα-tripeptides **9a–c** (20 μM each) across EYPC-LUVs⊃HPTS (**A**). Concentration- dependent ion-transport activity of **9b** (0–45 μM) across EYPC-LUVs⊃HPTS (**B**).

active compound **9b** (Figure 4B), the effective concentration required to reach 50% activity (i.e. EC_{50} = 15.83 ± 0.66 μM) was calculated (Figure S44).

The prominent ion-transport activity of **9b** encouraged us to investigate its ion-selectivity sequences. The observed intra-vesicular pH change is conjectured via antiport mechanism (i.e., A^-/OH^- or M^+/H^+ exchange). Therefore, the cation selectivity (Figure 5A) across the EYPC-LUVs containing HPTS dye was assessed in the presence of intravesicular NaCl against the extra-vesicular iso-osmolar monovalent metal chlorides (i.e., MCl where M^+ = Li^+, Na^+, K^+, Rb^+, and Cs^+). Similarly, the

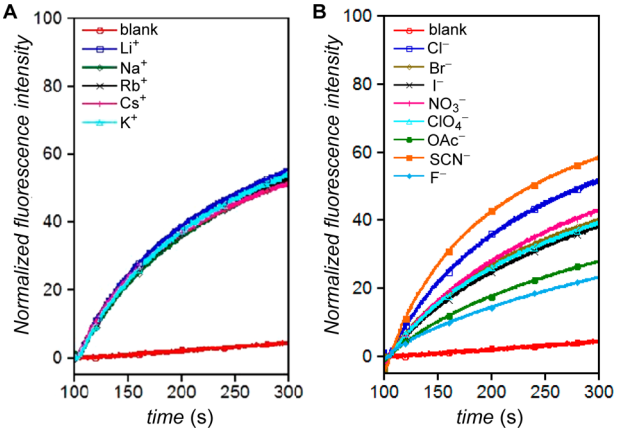

Figure 5. Ion-transport activity of **1b** (c = 20 μM) across EYPC-LUVs⊃HPTS determined by varying cations (**A**) and anions (**B**) in the extravesicular buffer.

anion selectivity (Figure 5B) sequence was determined by monitoring the pH change in the presence of extravesicular buffer containing iso-osmolar NaA (where A^- = F^-, Cl^-, Br^-, I^-, NO_3^-, SCN^-, AcO^- and ClO_4^-). The variation of external cations did not display any change in transport activity, indicating that **9b** is not selective toward the alkali cations. However, upon varying the extravesicular anion, significant change in transport behavior was observed with the following selectivity sequence: $SCN^- > Cl^- > NO_3^- \sim Br^- \sim I^- \sim ClO_4^- > AcO^- > F^-$. The anion selectivity of the compound during ion transport can be correlated to the NH···A^- interactions.

CONCLUSION

In conclusion, we studied the conformational aspects of the fluorinated-furanoid sugar containing acyclic tripeptides **9a–c** and compared them with their nonfluorinated analogues **15a–c**. The nonfluorinated tripeptides **15a–c** showed random conformation, whereas the tripeptides containing a fluorine substituent (**9a–c**) adopt a reverse-turn U-shaped conformation that is stabilized by the presence of fluorine without the participation of usual (NH···O═C) intramolecular H-bond. The tripeptides **9a**, **b** aggregate into the extended self-assembled helical stacks leading to the formation of nanorods. The self-assembled antiparallel fibers constructed from these tripeptides facilitate selective anion transport across the large unilamellar vesicles. The design of this novel class of motif will provide a powerful tool for the development of nanomaterial and biomaterial applications via supramolecular architecture.

EXPERIMENTAL SECTION

General Methods. All reactions were carried out with distilled and dried solvents using oven-dried glassware. ^{1}H NMR (300 MHz/400 MHz/500 MHz), ^{13}C NMR (75 MHz/100 MHz/125 MHz), COSY, NOESY (500 MHz), and HOESY (400 MHz) were recorded in $CDCl_3$. Chemical shifts are reported in δ units (parts per million) with reference to TMS as an internal standard. Melting points are uncorrected. Optical rotations were measured on a digital polarimeter with sodium light (589.3 nm) at 25 °C. High-resolution mass spectra (HRMS) were obtained in positive- ion electron-spray ionization (ESI) mode using a TOF (time-of-flight) analyzer. Thin layer chromatography was performed on precoated plates (0.25 mm, silica gel 60 F254). Column chromatography was carried out with silica gel (100–200 mesh). IR spectra were recorded on FTIR spectrophotometer as a thin film or using KBr pellets and reported in cm^{-1}. After neutralization, workup involves the washing of the combined organic layer with aqueous sodium bicarbonate, water, and brine, drying over anhydrous sodium sulfate, and evaporation of solvent under reduced pressure. Circular dichroism (CD) was performed using a cell of 2 mm path length. Spectra were recorded as an accumulation of three scans using a scan speed of 100 nm/min, with resolution of 1.0 nm, bandwidth of 1.0 nm, and a response of 1 s. Spectra were smoothed (5) and plotted using Origin Pro 6.0 software. Morphological images were recorded on an FESEM instrument.

Experimental Procedures. *(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-1,2;5,6-di-O-isopropylidene-α-D-gluco-1,4-furanose (3).* Compound **2** (10.0 g, 26.52 mmol) and anhydrous KF (15.4 g, 265.25 mmol) were dissolved in *t*-BuOH (200 mL) with continuous stirring. To this solution, 18-crown-6 ether (5 mol %), DBU (24.18 g, 158.82 mmol) and Val-OMe·HCl salt (5.82 g, 34.43 mmol) was added sequentially. The reaction mixture was stirred at room temperature for 2 h. After completion of reaction, the organic layer was evaporated under reduced pressure and extracted with ethyl acetate (200 mL). The reaction mixture was washed with saturated NH_4Cl (100 mL), 10% $NaHCO_3$ (100 mL) solution and water. The workup and purification by column chromatography on silica gel using ethyl acetate/hexane (1:4) gave **3** as a white crystalline solid (5.8 g, 52%

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yield): R_f = 0.5; (EtOAc/hexane = 3:7); mp = 78−80 °C; $[\alpha]_D^{25}$ = +43.4 (*c* = 0.1, MeOH); IR (KBr): ν_{max} = 3450−3300, 1743, 1697 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.01(dd, *J* = 8.1 and 2.3 Hz, 1H, NH-val), 6.00 (d, *J* = 3.8 Hz, 1H, H-1), 4.67 (dd, *J* = 20.2 and 7.7 Hz, 1H, H-4), 4.65 (dd, *J* = 14.7 and 3.8 Hz, 1H, H-2) 4.56 (dd, *J* = 8.1 and 4.7 Hz, 1H, valH$_\alpha$), 4.30−4.18 (m, 1H, H-5), 4.09 (dd, *J* = 8.8 and 6.2 Hz, 1H, H-6), 4.02 (dd, *J* = 8.8 and 4.7 Hz, 1H, H-6), 3.75 (s, 3H, OCH_3), 2.25−2.09 (m, 1H, valH$_\beta$), 1.58 (s, 3H), 1.35 (s, 3H), 1.34 (s, 3H), 1.30 (s, 3H), 0.96 (d, *J* = 6.7 Hz, 3H, val-CH_3), 0.94 (d, *J* = 6.4 Hz, 3H, val-CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.8, 164.1 (d, *J* = 21.5 Hz), 114.0, 109.9, 105.2, 100.6 (d, *J* = 194.8 Hz), 85.1 (d, *J* = 37.9 Hz), 81.7 (d, *J* = 19.9 Hz), 72.1 (d, *J* = 6.1 Hz), 66.8 (d, *J* = 1.6 Hz), 57.1 52.1, 31.4, 27.0, 26.7, 26.1, 25.3, 18.8, 18.0; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{19}H_{30}FNO_8Na$, 442.1853, found 442.1851.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-1,2-O-isopropylidene-α-D-gluco-1,4-furanose (**4**).* Compound **3** (4.8 g, 12.65 mmol) was dissolved in 60% acetic acid, (50 mL) and the reaction mixture was stirred at 55 °C for 3 h. The solvent was removed under vacuum, and the crude product was purified by column chromatography on silica gel using ethyl acetate/hexane (3:7) to give **4** as a thick liquid (4.0 g, 92% yield): R_f = 0.25, (EtOAc/hexane = 1:1); $[\alpha]_D^{25}$ = +56.4 (*c* = 0.1, MeOH); IR (neat): ν_{max} 3427 (br), 1739, 1685 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.19(dd, *J* = 8.2 and 1.6 Hz, 1H, NH-val), 6.01 (d, J = 3.8 Hz, 1H, H-1), 4.68 (dd, *J* = 13.6 and 3.8 Hz, 1H, H-2), 4.59 (dd, *J* = 27.1 and 8.7 Hz, 1H, H-4), 4.55 (dd, *J* = 8.2 and 4.8 Hz, 1H, valH$_\alpha$), 4.01−3.93 (m, 1H, H-5), 3.83 (dd, *J* = 11.6 and 3.2 Hz, 1H, H-6), 3.76 (s, 3H, OCH_3), 3.71 (dd, *J* = 11.6 and 5.2 Hz, 1H, H-6), 3.45−3.10 (br, 1H, OH), 2.50−2.30 (br, 1H, OH), 2.30−2.15 (m, 1H valH$_\beta$), 1.60 (s, 3H), 1.35 (s, 3H), 0.98 (d, *J* = 6.8 Hz, 3H, val-CH_3), 0.94 (d, *J* = 6.8 Hz, 3H, val-CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.0, 165.5 (d, *J* = 22.1 Hz), 114.2, 105.2, 100.0 (d, *J* = 194.0 Hz), 84.3 (d, *J* = 38.1 Hz), 81.0 (d, *J* = 20.3 Hz), 68.3 (d, *J* = 6.0 Hz), 63.8, 57.2, 52.4, 31.1, 26.9, 26.7, 18.9, 17.8; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{16}H_{26}FNO_8Na$, 402.1540, found 402.1541.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-1,2-O-isopropylidene-α-D-xylo-1,4-furanose (**5**).* To a stirred solution of **4** (4.0 g, 10.54 mmol) in acetone/water (40 mL, 8:2), cooled at 0 °C, $NaIO_4$ (2.25 g, 10.54 mmol) was added. After 30 min, the reaction mixture was allowed to attain rt, stirred for 3 h, filtered through Celite, and concentrated. The reaction mixture was extracted with ethyl acetate (20 mL × 3) and worked up to give crude product (3.4 g, 10.54 mmol) that was dissolved in methanol/water (20 mL, 9:1) at 0 °C. To this cooled solution, $NaBH_4$ was added (0.37 g, 9.78 mmol) and stirred for 20 min. The reaction mixture was neutralized by sat. NH_4Cl (10 mL), and the solvent was removed under reduced pressure and extracted with ethyl acetate (30 mL × 3). The workup and purification by column chromatography on silica gel using ethyl acetate/hexane (3:7) afforded **5** as a thick liquid (3 g, 81% yield, over two steps): R_f = 0.5, (EtOAc/hexane = 1:1); $[\alpha]_D^{25}$ = +104.0 (*c* = 0.15, MeOH); IR (neat): ν_{max} 3375 (br), 1742, 1688 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.16(bd, *J* = 8.0 Hz, 1H, NH-val), 6.03 (d, *J* = 3.6 Hz, 1H, C1H), 4.75−4.65 (m, 1H, C2H,C4H), 4.57 (dd, *J* = 8.0 and 4.7 Hz, 1H, Val*α*H), 3.93−3.83 (m, 2H, C5H), 3.75 (s, 3H, OMe), 2.54−2.35 (br s, 1H, OH), 2.27−2.17 (m, 1H, Val*α*H), 1.60 (s, 3H, CH_3), 1.37 (s, 3H, CH_3), 0.98 (d, *J* = 6.8 Hz, 3H, CH_3 Val), 0.94 (d, *J* = 6.9 Hz, 3H, CH_3 Val).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.9, 164.9 (d, *J* = 22.3 Hz), 114.0, 105.1, 92.7 (d, *J* = 192.9 Hz), 84.4 (d, *J* = 37.7 Hz), 81.5 (d, *J* = 19.9 Hz), 58.7 (d, *J* = 8.2 Hz), 57.1, 52.4, 31.1, 26.9, 26.6, 18.9, 17.7; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{24}FNO_7Na$, 372.1435, found 372.1436.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-5-deoxy-5-azido-1,2-O-isopropylidene-α-D-xylo-1,4-furanose (**6**).* To a cooled solution of **5** (3.0 g, 8.58 mmol) in dichloromethane (30 mL) and pyridine (2.07 mL, 25.76 mmol), triflic anhydride (2.13 mL, 12.87 mmol) was added under a nitrogen atmosphere, and the reaction mixture was stirred at 10 °C. After 1 h, water (10 mL) was added and the solution was extracted with dichloromethane (30 mL x 3). The workup gave a crude product (4.1 g, 8.59 mmol) that was dissolved in dry DMF (15 mL). To this solution, TBAI (0.01 mmol) and sodium azide (1.66 g, 25.54 mmol) was added at rt, and the reaction mixture was heated at 70 °C for 2 h. The reaction mixture was concentrated under vacuum, diluted with water (20 mL), and extracted with ethyl acetate (20 mL x 3). The workup and purification by column chromatography on silica gel (100−200 mesh) using ethyl acetate/hexane (1:9) gave **6** as a white solid (2.5 g, 77% yield): R_f = 0.5 (EtOAc/hexane = 1:4); mp = 82−84 °C; $[\alpha]_D^{25}$ = 23.33 (*c* = 0.14, MeOH); IR (KBr): ν_{max} = 3367, 2974, 2102, 1734, 1682 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 6.90 (bd, *J* = 6.90 Hz, 1H, NH-val), 6.07 (d, *J* = 3.39 Hz, 1H, H-1), 4.90−4.73 (m, 1H, H-4), 4.66 (dd, *J* = 14.9 and 3.4, Hz, 1H, H-2), 4.56 (dd, *J* = 6.9 and 4.7 Hz, 1H, valH$_\alpha$), 3.76 (s, 3H, OMe), 3.58 (dd, *J* = 12.6 and 7.2 Hz, 1H, H-5), 3.43 (dd, *J* = 12.6 and 4.8 Hz, 1H, H-5), 2.30−2.15 (m, 1H, valH$_\beta$), 1.59 (s, 3H), 1.36 (s, 3H), 0.98 (d, *J* = 6.9 Hz, 3H), 0.94 (d, *J* = 6.8 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.7, 163.8 (d, *J* = 21.9 Hz), 114.1, 105.2, 100.3 (d, *J* = 195.2 Hz), 84.6 (d, *J* = 37.3 Hz), 79.9 (d, *J* = 19.5 Hz), 57.1, 52.4, 48.2 (d, *J* = 8.0 Hz), 31.2, 26.9, 26.6, 18.9, 17.77; HRMS (ESI-TOF) *m*/*z*: [M + Na]$^+$ calcd for $C_{15}H_{23}FN_4O_6Na$ 397.1494, found, 397.1492.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-5-deoxy-5-amino-1,2-O-isopropylidene-α-D-xylo-1,4-furanose (**7**).* A solution of **6** (2.5 g, 7.17 mmol) in methanol (15 mL) and 10% Pd/C (0.74 g, 2.09 mmol) was hydrogenated under balloon pressure at rt for 2 h. The solution was filtered through Celite, and the Celite bed was washed with methanol (10 mL). The combined solution was concentrated under reduced pressure to give 7 as thick liquid (2.1 g, 90% yield): R_f = 0.3, (EtOAc/MeOH = 9.5:0.5); $[\alpha]_D^{25}$ = −6.66 (*c* = 0.10, MeOH); IR (neat): ν_{max} = 3423 (br), 3373 (br), 2966, 1741, 1685 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 7.19 (bd, *J* = 7.8 Hz, 1H, NH-val), 6.01 (d, *J* = 3.8 Hz, 1H, H-1), 4.70−4.58 (m, 3H, H-2, H-4 and ValH$_\alpha$), 3.75 (s, 3H, OMe), 3.08−2.93 (m, 2H, H-5), 2.30−2.15 (m, *J* = 6.7, 1H, valH$_\beta$), 1.91 (br, 2H, NH_2, exchanges with D_2O), 1.58 (s, 3H), 1.35 (s, 3H), 0.97 (d, *J* = 6.8, 3H), 0.94 (d, *J* = 6.9, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 171.2, 164.9 (d, *J* = 21.9 Hz), 113.8, 104.9, 100.6 (d, *J* = 193.6 Hz), 85.0 (d, *J* = 37.2 Hz), 82.7 (d, *J* = 21.9 Hz), 57.3, 52.4, 39.5 (d, *J* = 7.8 Hz), 31.2, 27.0, 26.7, 19.0, 17.9; HRMS (ESI-TOF) *m*/*z*: [M + H]$^+$ calcd for $C_{15}H_{26}FN_2O_6$ 349.1769, found, 349.1764.

General Acid-Amine Coupling Procedure for the Synthesis of 9a, 9b, and 9c. To the stirred solution of compound 7 (1 mmol) and DIPEA (1 mmol) in dry CH_3CN under a nitrogen atmosphere at 0 °C, BocNH-Ala-OH/BocNH-Leu-OH/BocNH-Phe-OH (1 mmol) was added, and the solution was stirred for 5 min. To this cooled solution, HATU (1 mmol) was added, and the reaction was allowed to attain rt and stirred for 5 h. The reaction mixture was diluted with EtOAc (100 mL), and the organic layer was washed with 1 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine. The workup gave a crude product that was purified by column chromatography on silica gel using EtOAc/hexane = 4:1 to give tripeptide **9a**/**9b**/**9c**, respectively, as a white crystalline solid.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-5-deoxy-5-N-(L-Ala-NHBoc)-1,2-O-isopropylidene-α-D-xylo-1,4-furanose (**9a**).* White solid, 0.6 g, 80% yield: R_f = 0.6 (EtOAc/hexane = 2:3); mp = 145−147 °C; $[\alpha]_D^{25}$ = 28.66 (*c* = 0.14, MeOH); IR (KBr): ν_{max} = 3449, 3321, 3080, 2980, 1757, 1701, 1670 cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ = 6.93 (dd, *J* = 8.1 and 2.5 Hz, 1H, Val-NH), 6.44 (bs, 1H, NH-5), 6.01 (d, *J* = 3.8 Hz, 1H, H-1), 5.13 (bs, 1H, AlaNH), 4.75−4.63 (m, 2H, H-2, H-4), 4.52 (dd, *J* = 8.1 and 4.6 Hz, 1H, ValH$_\alpha$), 4.22−4.10 (m, 1H, AlaH$_\alpha$), 3.80 (s, 3H, OMe), 3.73−3.47 (m, 2H, H-5), 2.30−2.19 (m, 1H, ValH$_\beta$), 1.56 (s, 3H), 1.44 (s, 9H, 3 × CH_3), 1.34 (s, 3H, CH_3), 1.29 (d, *J* = 7.0 Hz, 3H, Ala-CH_3), 0.98 (d, *J* = 6.8, 3H, Val-CH_3), 0.94 (d, *J* = 6.9, 3H, Val-CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.8, 172.1, 164.4 (d, *J* = 21.1 Hz), 155.3, 114.0, 104.9, 100.2 (d, *J* = 195.0 Hz), 84.8 (d, *J* = 37.6 Hz), 79.3 (d, *J* = 19.6 Hz), 57.3, 52.5, 49.9, 36.4 (d, *J* = 8.2 Hz), 30.7,

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29.7, 28.3, 26.9, 26.6, 19.0, 18.9, 17.8; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{23}H_{38}FN_3O_9Na$ 542.2484, found, 542.2483.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-5-deoxy-5-N-(L-Leu-NHBoc)-1,2-O-isopropylidene-α-D-xylo-1,4-furanose (**9b**).* White solid, 0.58 g, 72% yield: R_f = 0.6 (EtOAc/hexane = 2:3); mp = 115−117 °C; $[\alpha]_D^{25}$ = −44.33 (c = 0.14, MeOH); IR (KBr): ν_{max} 3449, 3321, 3070, 2957, 1743, 1701, 1659 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.97 (dd, J = 8.2 and 1.8 Hz, 1H, Val-NH), 6.40 (bs, 1H, NH-5), 6.02 (d, J = 3.8 Hz, 1H, H-1), 4.94 (bs, 1H, LeuNH), 4.70 (dt, J = 27.0 and 6.24 Hz, 1H, H-4), 4.66 (dd, J = 14.4 and 3.8 Hz, 1H, H-2), 4.54 (dd, J = 8.2 and 4.8, Hz, 1H, ValH$_\alpha$), 4.18−4.00 (m, 1H, LeuH$_\alpha$), 3.79 (s, 3H, OMe), 3.72−3.45 (m, 2H, H-5), 2.32−2.17 (m, 1H, ValH$_\beta$), 1.87−1.60 (m, 3H, LeuH$_\beta$, H$_\gamma$), 1.56 (s, 3H), 1.44 (s, 9H, 3xCH$_3$), 1.35 (s, 3H), 1.03−0.85 (m, 12H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.7, 171.9, 164.3 (d, J = 24.0 Hz), 155.6, 114.0, 104.9, 100.2 (d, J = 195.00 Hz), 84.7 (d, J = 37.5 Hz), 79.9, 79.3 (d, J = 19.5 Hz), 57.3, 52.9, 52.5, 41.3, 36.7 (d, J = 8.0 Hz), 30.9, 28.3, 26.9, 26.6, 24.7, 23.1, 21.8, 18.9, 17.8; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{26}H_{44}FN_3O_9Na$ 584.2954, found, 584.2963.

*(3S)-Carbonyloxy-[L-Val-OMe]-3-deoxy-3-fluoro-5-deoxy-5-N-(L-Phe-NHBoc)-1,2-O-isopropylidene-α-D-xylo-1,4-furanose (**9c**).* White solid, 0.62 g, 72% yield: R_f = 0.65 (EtOAc/hexane = 2:3); mp = 103−105 °C; $[\alpha]_D^{25}$ = −28.66 (c = 0.15, MeOH); IR (KBr): ν_{max} 3346 (br), 3063, 2968, 2930, 1736, 1687, 1672 cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35−7.15 (m, 5H, Ph), 6.93 (dd, J = 8.2 and 2.4 Hz, 1H, Val-NH), 6.22 (bt, J = 5.3 Hz, 1H, NH-5), 5.99 (d, J = 3.9 Hz, 1H, H-1), 5.15−5.01 (bs, 1H, PheNH), 4.67 (dt, J = 31.2 and 7.3 Hz, 1H, H-4), 4.65 (dd, J = 14.6 and 3.9 Hz, 1H, H-2), 4.54 (dd, J = 8.2 and 4.7 Hz, 1H, ValH$_\alpha$), 4.42−4.25 (br, 1H, PheH$_\alpha$), 3.76 (s, 3H, OMe), 3.70−3.55 (m, 1H, H-5), 3.56−3.40 (m, 1H, H-5), 3.08 (dd, J = 13.9 and 6.2 Hz, 1H, PhCH$_2$), 2.97 (dd, J = 13.9 and 7.0 Hz, 1H, PhCH$_2$), 2.33−2.15 (m, 1H, ValH$_\beta$), 1.56 (s, 3H), 1.38 (s, 9H, 3xCH$_3$), 1.34 (s, 3H), 0.98 (d, J = 6.8 Hz, 3H), 0.94 (d, J = 6.9 Hz, 3H).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.1, 171.5, 164.5 (d, J = 20.5 Hz), 155.5, 137.0, 129.5, 128.7, 126.9, 114.1, 105.1, 100.3 (d, J = 194.8 Hz), 84.9 (d, J = 37.4 Hz), 80.1, 79.4 (d, J = 19.6 Hz), 57.4, 55.6, 52.6, 38.6, 36.8 (d, J = 8.3 Hz), 31.0, 28.4 (strong), 27.0, 26.7, 19.1, 17.9; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{29}H_{42}FN_3O_9Na$, 618.2797, found 618.2801.

*(3R)-Carbonyloxy-[L-Val-OMe]-3-deoxy-5-O-tert-butyldimethylsilyl-1,2-O-isopropylidene-α-D-ribo-1,4-furanose (**12**).* To a cooled (0 °C) solution of compound **11** (2.5 g, 7.84 mmol) in CH_3CN/H_2O (1:1), BAIB (5.3 g, 16.8 mmol), $NaHCO_3$ (0.98 g, 11.77 mmol), and TEMPO (0.18 g, 1.17 mmol) were sequentially added. The reaction mixture was allowed to attain rt and stirred for 6 h. The reaction was quenched by aqueous $Na_2S_2O_3$. The solvent was removed under reduced pressure and extracted with ethyl acetate (50 mL × 3). The workup afforded a thick liquid (2.6 g, 7.47 mmol) that was dissolved in CH_3CN (5 mL) and cooled to 0 °C. To this, a solution of HCl·H_2N-Val-OMe (2.52 g, 1.94 mmol) and DIPEA (3.82 mL, 22.42 mmol) in CH_3CN (20 mL), followed by HATU (3.12 g, 8.22 mmol) was added, and the reaction mixture was stirred at rt for 5 h. The organic layer was evaporated under reduced pressure and extracted with ethyl acetate (50 mL × 3), washed with 1 N HCl (50 mL), 10% aq $NaHCO_3$ (50 mL), and water. The workup and purification by column chromatography on silica gel using ethyl acetate/hexane (1.5:9) gave **12** as a thick liquid (2.2 g, 63% yield over 2 steps): R_f = 0.8, (EtOAc/hexane = 3:7); $[\alpha]_D^{20}$ = +92.0 (c = 0.13, $CHCl_3$); IR (neat): ν_{max} = 3500−3250 (br), 2956, 2931, 1745, 1710, 1650, 1531 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.93(d, J = 8.5 Hz, 1H, NH-val), 5.85 (d, J = 3.7 Hz, 1H, H-1), 4.86 (dd, J = 4.8 and 3.7 Hz, 1H, H-2), 4.59 (dd, J = 8.5 and 4.7 Hz, 1H, valH$_\alpha$), 4.35 (ddd, J = 10.1, 3.4, and 2.3 Hz, 1H, H-4), 3.97 (dd, J = 11.6 and 2.3 Hz, 1H, Ha-5), 3.80 (dd, J = 11.6 and 3.4 Hz, 1H, Hb-5), 3.75 (s, 3H, OMe), 2.97 (dd, J = 10.1 and 4.8 Hz, 1H, H-3), 2.22−2.15 (m, 1H, valH$_\beta$), 1.56 (s, 3H, CH$_3$), 1.37 (s, 3H, CH$_3$), 0.94 (d, J = 6.8 Hz, 3H, Val-CH$_3$), 0.90 (d, J = 6.8 Hz, 3H, Val-CH$_3$), 0.88 (s, 9H, 3 × CH$_3$), 0.06 (s, 3H, CH$_3$), 0.05 (s, 3H, CH$_3$).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.4, 167.6, 112.6, 105.5, 81.0, 80.4, 62.6, 56.9, 52.1, 49.8, 31.4, 26.8, 26.6, 25.9 (strong), 19.0, 18.4 (w), 17.6, 5.2, 5.3; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{21}H_{39}NO_7SiNa$, 468.2388, found 468.2400.

*(3R)-Carbonyloxy-[L-Val-OMe]-3-deoxy-1,2-O-isopropylidene-α-D-ribo-1,4-furanose (**13**).* To a cooled solution (0 °C) of compound **12** (2 g, 4.8 mmol) in dry THF (20 mL), 1 M TBAF in THF (10 mL, 7.2 mmol) was added, and the reaction mixture was stirred at rt. After 1 h, the reaction was quenched with aq ammonium chloride solution and extracted with ethyl acetate (25 mL × 3). The workup and purification by column chromatography on silica gel using ethyl acetate gave **13** as a thick liquid (1.2 g, 81% yield): R_f = 0.4, (EtOAc: MeOH = 1:0.1); $[\alpha]_D^{20}$ = +78.0 (c = 0.18, $CHCl_3$); IR (neat): ν_{max} = 3650−3150 (br), 2964, 1737, 1660, 1539 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 7.22(d, J = 8.3 Hz, 1H, NH-val), 5.89 (d, J = 3.7 Hz, 1H, H-1), 4.92 (t, J = 4.2 Hz, 1H, H-2), 4.58 (dd, J = 8.3 and 4.8 Hz, 1H, valH$_\alpha$), 4.33 (dt, J = 10.3 and 4 Hz, 1H, H-4), 3.89 (dd, J = 12 and 4 Hz, 1H, Ha-5), 3.83 (bd, J = 12 Hz, 1H, Hb-5), 3.75 (s, 3H, OMe), 2.95 (bs, 1H, exchenges with D_2O, OH), 2.91 (dd, J = 10.3 and 4.2 Hz, 1H, H-3), 2.22−2.19 (m, 1H, valH$_\beta$), 1.57 (s, 3H, CH$_3$), 1.39 (s, 3H, CH$_3$), 0.94 (d, J = 6.8 Hz, 3H, Val-CH$_3$), 0.90 (d, J = 6.8 Hz, 3H, Val-CH$_3$).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.2, 168.1, 112.9, 105.3, 80.0, 79.9, 62.6, 57.1, 52.3, 51.7, 31.2, 26.7, 26.5, 19.0, 17.6; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{15}H_{25}NO_7Na$, 354.1523, found 354.1528.

*(3R)-Carbonyloxy-[L-Val-OMe]-3,5-dideoxy-5-azido-1,2-O-isopropylidene-α-D-ribo-1,4-furanose (**14**).* To compound **13** (1.0 g, 3.01 mmol) in pyridine (10 mL) at rt under a nitrogen atmosphere DMAP (0.036 g, 0.3 mmol) was added, followed by TsCl (0.575 g, 3.01 mmol), and the reaction mixture was stirred at 70 °C for 4 h. The workup gave a thick liquid (1.4 g, 2.88 mmol) that was dissolved in dry DMF (10 mL). To this solution, TBAI (0.01 mmol) and sodium azide (0.93 g, 14.4 mmol) were added at rt, and the reaction mixture was heated at 110 °C. After 4 h, the reaction mixture was cooled to rt and concentrated under vacuum, diluted with water (20 mL), and extracted with ethyl acetate (20 mL × 3). The workup and purification by column chromatography on silica gel (100−200 mesh) using ethyl acetate/hexane (3:7) gave **14** as a thick liquid (0.5 g, 46% yield): R_f = 0.8 (EtOAc/hexane = 7:3); $[\alpha]_D^{20}$ = +81.0 (c = 0.10, $CHCl_3$); IR (neat): ν_{max} = 3400−3200, 2964, 2102, 1741, 1656, 1535 cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$): δ = 6.92(d, J = 8.3 Hz, 1H, NH-val), 5.92 (d, J = 3.7 Hz, 1H, H-1), 4.90 (t, J = 4.2 Hz, 1H, H-2), 4.57 (dd, J = 8.5 and 4.7 Hz, 1H, valH$_\alpha$), 4.45 (ddd, J = 10.3, 4.2, and 2.5 Hz, 1H, H-4), 3.79 (dd, J = 13.4 and 2.5 Hz, 1H, Ha-5), 3.75 (s, 3H, OMe), 3.47 (dd, J = 13.4 and 4.2 Hz, 1H, Hb-5), 2.91 (dd, J = 10.3 and 4.2 Hz, 1H, H-3), 2.20−2.17 (m, 1H, valH$_\beta$), 1.56 (s, 3H, CH$_3$), 1.38 (s, 3H, CH$_3$), 0.94 (d, J = 6.8 Hz, 3H, Val-CH$_3$), 0.91 (d, J = 6.8 Hz, 3H, Val-CH$_3$).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.3, 166.9, 112.9, 105.4, 79.8, 78.9, 56.9, 52.3, 52.0, 50.9, 31.3, 26.7, 26.5, 19.0, 17.6; HRMS (ESI-TOF) m/z: [M + Na]$^+$ calcd for $C_{15}H_{24}N_4O_6Na$, 379.1588, found 379.1599.

General Acid-Amine Coupling Procedure for the Synthesis of 15a, 15b, and 15c. The azide compound **14** (0.4 g, 1.12 mmol) in methanol (5 mL) and 10% Pd/C (0.03 g, 0.28 mmol) was hydrogenated under balloon pressure at rt for 3 h. The solution was filtered through Celite, and the Celite bed was washed with methanol (10 mL). The combined solution was concentrated under reduced pressure to give a thick liquid of a crude amine compound (0.35 g). To this crude amine (1 mmol), DIPEA (1 mmol) in dry CH_3CN under nitrogen atmosphere, BocNH-Ala-OH/BocNH-Leu-OH/BocNH-Phe-OH (1 mmol) was added at 0 °C, and the solution was stirred for 5 min. To this cooled solution, CMPI (1 mmol) was added, and the reaction was allowed to attain rt and was stirred for 5 h. The reaction mixture was diluted with EtOAc (100 mL) and the organic layer was washed with 1 M HCl (50 mL), saturated aqueous $NaHCO_3$ (50 mL), and brine. The workup gave a crude product that was purified by column chromatography on silica gel using EtOAc/hexane

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= 7:3 to give the nonfluorinated tripeptides **15*a*/15*b*/15c**, respectively, as a foamy solid.

*(3R)-Carbonyloxy-[L-Val-OMe]-3,5-dideoxy-5-N-(L-Ala-NHBoc)-1,2-O-isopropylidene-α-D-ribo-1,4-furanose (**15a**).* Foamy solid, 0.046 g, 70% yield: R_f = 0.5 (EtOAc); $[\alpha]_D^{20}$ = +13.8 (*c* = 0.12, $CHCl_3$); IR (neat): ν_{max} = 3500−3200 (br), 2978, 1739, 1680, 1662 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.18 (d, *J* = 8.3 Hz, 1H, NH (III)), 6.76 (bs, 1H, NH (II)), 5.83 (d, *J* = 3.5 Hz, 1H, H-1), 5.11 (d, *J* = 6.6 Hz, 1H, NH (I)), 4.87 (t, *J* = 4.3 Hz, 1H, H-2), 4.56 (dd, *J* = 8.3 and 4.9 Hz, 1H, $ValH_\alpha$), 4.22−4.36 (m, 1H, H-4), 4.20−4.11 (m, 1H, $AlaH_\alpha$), 3.75 (s, 3H, OMe), 3.74−3.66 (m, 1H, Ha-5), 3.61−3.51 (m, 1H, Hb-5), 2.69 (dd, *J* = 10.1 and 4.3 Hz, 1H, H-3), 2.22−2.18 (m, 1H, $ValH_\beta$), 1.54 (s, 3H, CH_3), 1.44 (s, 9H, 3 × CH_3), 1.35 (s, 3H, CH_3), 1.34 (d, *J* = 7.2 Hz, 3H, Ala-CH_3), 0.96 (d, *J* = 6.8, 3H, Val-CH_3), 0.92 (d, *J* = 6.8, 3H, Val-CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 173.0, 172.4, 167.3, 155.5, 112.8, 105.2, 80.4, 80.1 (w), 78.2, 57.2, 52.4, 52.2, 50.4, 41.0, 31.1, 28.3 (s), 26.7, 26.5, 19.0, 18.6, 17.7; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{23}H_{39}N_3O_9Na$, 524.2579, found 524.2584.

*(3R)-Carbonyloxy-[L-Val-OMe]-3,5-dideoxy-5-N-(L-Leu-NHBoc)-1,2-O-isopropylidene-α-D-ribo-1,4-furanose (**15b**).* Foamy solid, 0.053 g, 75% yield: R_f = 0.7 (EtOAc); $[\alpha]_D^{20}$ = +76.4 (*c* = 0.10, $CHCl_3$); IR (neat): ν_{max} = 3500−3100 (br), 2960, 3080, 1741, 1662, 1531 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.19 (d, *J* = 8.0 Hz, 1H, NH (III)), 6.75 (t, *J* = 5.6 Hz, 1H, NH (II)), 5.83 (d, *J* = 3.5 Hz, 1H, H-1), 4.94 (d, *J* = 6.6 Hz, 1H, NH (I)), 4.87 (dd, *J* = 4.4 and 3.5 Hz, 1H, H-2), 4.56 (dd, *J* = 8.0 and 4.8 Hz, 1H, $ValH_\alpha$), 4.53−4.35 (m, 1H, H-4), 4.15−4.05 (m, 1H, $LeuH_\alpha$), 3.75 (s, 3H, OMe), 3.73−3.55 (m, 2H, Ha-5, Hb-5), 2.68 (dd, *J* = 10.1 and 4.4 Hz, 1H, H-3), 2.22−2.17 (m, 1H, $ValH_\beta$), 1.70−1.60 (m, 2H, Leuβ $-CH_2$), 1.55 (s, 3H, CH_3), 1.50−1.40 (m, 1H, Leuγ-H), 1.44 (s, 9H, 3 × CH_3), 1.35 (s, 3H, CH_3), 1.0−0.9 (m, 12H, 4 × CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.9, 172.4, 167.3, 155.7, 112.8, 105.2, 80.4, 80.1 (w), 78.3, 57.2, 53.3, 52.4, 52.2, 41.4, 40.9, 31.0, 28.3 (s), 26.7, 26.5, 24.8, 23.0, 21.8, 19.0, 17.7; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{26}H_{45}N_3O_9Na$, 566.3048, found 566.3046.

*(3R)-Carbonyloxy-[L-Val-OMe]-3,5-dideoxy-5-N-(L-Phe-NHBoc)-1,2-O-isopropylidene-α-D-ribo-1,4-furanose (**15c**).* Foamy solid, 0.050 g, 70% yield: R_f = 0.8 (EtOAc); $[\alpha]_D^{20}$ = +55.6 (*c* = 0.13, $CHCl_3$); IR (neat): ν_{max} = 3500−3200, 2974, 1739, 1662, 1529, 756 cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$): δ = 7.33−7.20 (m, 5H, Ph), 6.98 (d, *J* = 8.3 Hz, 1H, NH (III)), 6.35 (bs, 1H, NH (II)), 5.65 (s, 1H, H-1), 5.10 (d, *J* = 6.3 Hz, 1H, NH (I)), 4.68 (bs, 1H, H-2), 4.57 (dd, *J* = 8.3 and 4.8 Hz, 1H, $ValH_\alpha$), 4.42−4.29 (m, 2H, H-4 and PheHα), 3.76 (s, 3H, OMe), 3.72−3.62 (m, 1H, Ha-5), 3.50−3.38 (m, 1H, Hb-5), 3.05 (bd, *J* = 6.7 Hz, 2H, $PhCH_2$), 2.27−2.16 (m, 2H, H-3 and $ValH_\beta$), 1.51 (s, 3H, CH_3), 1.40 (s, 9H, 3 × CH_3), 1.33 (s, 3H, CH_3), 0.96 (d, *J* = 6.8, 3H, Val-CH_3), 0.93 (d, *J* = 6.8, 3H, Val-CH_3).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 172.5, 171.4, 167.0, 155.3, 136.9, 129.4, 128.6, 126.9, 112.8, 105.0, 80.5, 80.1 (w), 77.9, 57.1, 56.0, 52.2, 51.5, 40.5, 38.6, 31.2, 28.3 (s), 26.7, 26.5, 19.0, 17.7; HRMS (ESI-TOF) *m*/*z*: $[M + Na]^+$ calcd for $C_{29}H_{43}N_3O_9Na$, 600.2892, found 600.2890.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.joc.7b00661.

NMR spectra of all new compound, detailed 2D-NMR investigations including complete ^1H NMR and NOESY assignment and details of CD, ESI-MS, SEM, DFT calculations, ion transport activity experiments etc.(PDF)

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Notes

The authors declare no competing financial interest.

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