

Self-Assembled Rigid-Rod Ionophores

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During the course of recent efforts to explore the scope and limitations of rigid-rod molecules as versatile biomimetics,¹ we have observed giant ion channels formed at high voltages in planar lipid bilayers with a lifetime of less than 0.1 ms (Figure 1A).^{1c} The proposed structure of this voltage-dependent ion channel, i.e., self-assembly **1ⁿ**, could not be further studied because of its transient nature, and design and synthesis of more stable analogues was essential to determine structure and activity of toroidal rigid-rod supramolecules. The use of interdigitating peptidic side chains to establish β -sheets between rigid-rod scaffolds (e.g., **2ⁿ**, Figure 1A) was particularly inviting because many biological toroidal supramolecules have been shown to consist of β -barrels,² and cylindrical β -barrels formed by self-assembly of synthetic circular peptides have been studied extensively.³ Most importantly, the feasibility to create supramolecular, antiparallel β -sheets by interdigitation has been demonstrated in an elegant model study,⁴ and the formation of β -sheets in, e.g., **2ⁿ** is further likely to occur with high cooperativity⁵ because of the preorganizing rigid-rod scaffold. It is further interesting to note that despite their functional versatility in biological systems, β -barrels have attracted little attention for biomimetic modeling of complex biomolecules such as ion channel proteins.^{1,3,6} Here we wish to report a model study to define the minimal requirements for β -sheet formation in-between rigid-rod scaffolds, focusing on self-assembled, presumably dimeric rigid-rod ionophore **2²** (Figure 1B).⁷

The rigid-rod octamers **2** and **3** were prepared from octa- and tetraanisoles **4**^{1a} and **5**^{1b} respectively (Scheme 1). While tetra-Leu **3** was readily prepared from tetraanisole **5** by aryl ether cleavage, conversion of tetraphenol **6** to tetraglycolate **7**, ester hydrolysis, and amide formation of the resulting tetraacid **8**, severe solubility problems with octamethylglycolate **9** required modifications of this sequence to prepare octa-Leu **2**. In sharp contrast to methylglycolate **9**, the corresponding *tert*-butyl ester **10** (obtained from octaphenol **11** and *tert*-butyl bromoacetate) exhibited

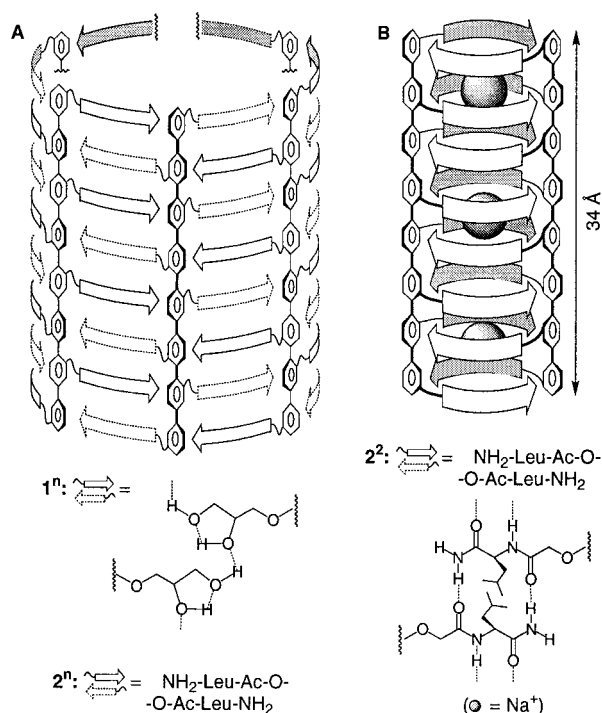
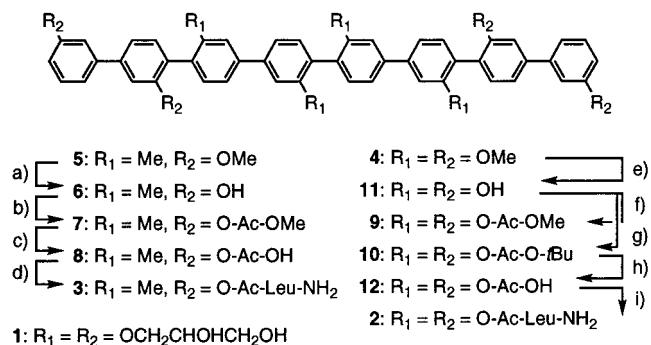


Figure 1. (A) Hypothetical toroidal rigid-rod supramolecules with lateral side chains containing vicinal diols (**1ⁿ**)^{1c} or peptides (**2ⁿ**). (B) Self-assembled dimer **2²**.

Scheme 1^a

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^a Key: (a) BBr₃; (b) methyl bromoacetate, Cs₂CO₃, 76% from **5**; (c) 1 M aqueous NaOH; (d) H-Leu-NH₂, EDC, HOBT, NEt₃, DMF, 68% from **7**; (e) BBr₃; (f) see footnote b, 55% from **4**; (g) *tert*-butyl bromoacetate, Cs₂CO₃, 62% from **4**; (h) TFA/CH₂Cl₂; (i) see footnote d, 14% from **10**.

excellent physical properties and could be cleaved under mild conditions to give octaacid **12**. However, the final rigid-rod octa-Leu **2** was again difficult to purify, presumably due to the ionophoric properties of its dimer **2²** (see below), and extensive exposure of the product to PTLC and RP-HPLC accounts for the low yield of the final step.

Although the oligomeric nature of **2** limited the applicability of NMR spectroscopy, 2D ¹H, ¹H-COSY NMR experiments (D₂O/CD₃OD) allowed us to assign the C(α)-H resonances at 4.32–4.52 ppm. Their downfield shift with respect to random coil values (4.17 ± 0.1 ppm)⁸ is indicative for, in this case, self-assembly by intermolecular β -sheet formation, because intramolecular β -sheet formation along the rigid-rod scaffold is sterically unlikely. The negative Cotton effect (CE) at 216 nm in the concentration-

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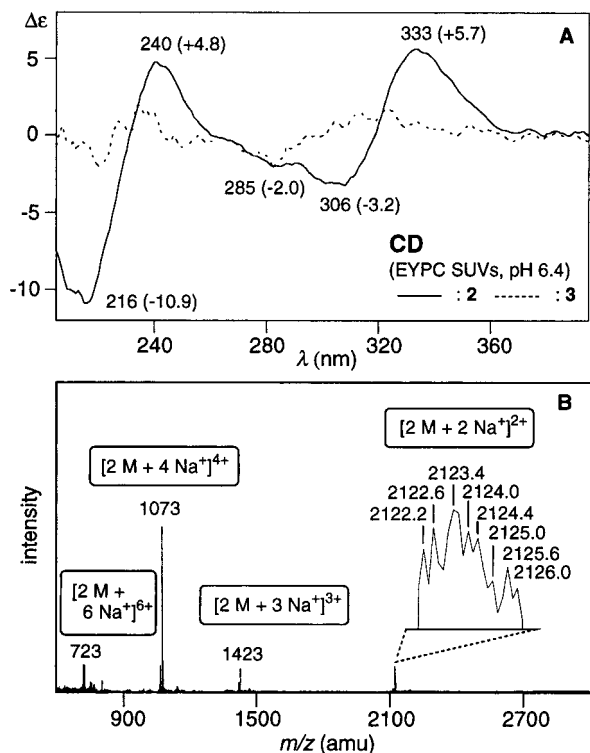


Figure 2. (A) CD spectra of octa-Leu **2** (solid) and tetra-Leu **3** (dashed). (B) ESI-MS of **2** (MeOH/0.1% HCOOH).

independent circular dichroism (CD) spectra of **2** further supported β -sheet formation and thus self-assembly in, e.g., water as well as egg yolk phosphatidylcholine (EYPC) bilayers.⁹ Bisignate CEs centered around the $^1L_a/{}^1L_b$ absorptions at 312 nm^{1a} implied exciton coupling, i.e., stereoselective organization of the rigid-rod chromophore (Figure 2A).¹⁰

In light of mounting evidence for the identity of gas-phase complexes seen in ESI-MS with those in solution,¹¹ the four major peaks for **2** strongly supported the presence of ionophoric dimers (2^2) and the absence of trimeric or higher oligomers in solution (2^n , Figure 2B). The increasingly poor resolution of the isotopic patterns with multiple charges, shown for the doubly charged peak around m/z 2123.4, is in full agreement with this conclusion. Tetra-Leu **3** was only measurable as monomer with negative detection mode.

Consistent with ESI-MS results, the absence of toroidal suprastructures with internal pores of diameters >5 Å (2^n) was further implied by the incapacity of octa-Leu **2** to mediate release of CF (a self-quenching, watersoluble fluorophore) across the lipid bilayer of uniformly sized EYPC-SUVs (SUV = small unilamellar vesicle).¹² However, when added to EYPC-SUVs having entrapped pH sensitive fluorophore 8-hydroxypyrene-1,3,6-trisulfonic acid (HPTS) and a transmembrane pH gradient, *nanomolar*

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(12) Dye leakage and ion transport experiments were performed as previously described.^{1a}

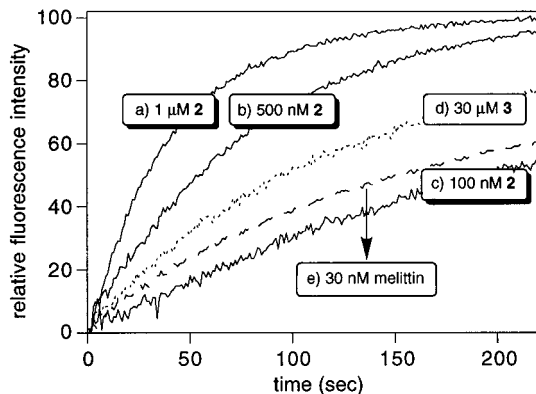


Figure 3. Change in fluorescent intensity of EYPC-SUV entrapped HPTS as a function of time after the addition of **2** (solid; curve a: 1 μ M, curve b: 500 nM, curve c: 100 nM), **3** (dotted; curve d: 30 μ M), and melittin (dashed; curve e: 30 nM) followed by 20 μ L of 0.5 M NaOH ($pH_{out} = 7.4$, $pH_{in} = 6.4$; 10 mM Na_2HPO_4/NaH_2PO_4 , 100 mM NaCl). Intravesicular pH was monitored ratiometrically [$I_t = I_a$ ($\lambda_{em} = 510$ nm, $\lambda_{ex} = 460$ nm)/ I_b ($\lambda_{em} = 510$ nm, $\lambda_{ex} = 405$ nm)] and normalized $\{(I_t - I_0)/(I_\infty - I_0) \times 100\}$. I_∞ was determined by adding 50 μ L of 1.2% Triton X-100.

concentrations were sufficient to mediate rapid intravesicular pH changes (Figure 3). Ion transport by **2** was >75 -times more and ~ 3 -times less efficient than that by **3** and the pore forming melittin, respectively. The linear dependence of transport rates with respect to the concentration of **2** is consistent with the incorporation of preformed dimer 2^2 as indicated by the spectroscopic data.

The ionophoric properties of **2**, implied by ESI-MS as well as transport experiments, were further corroborated by picrate extraction.⁷ Comparison of the relative concentrations of picrate and oligophenylene **2** after extraction of solid sodium picrate into $CDCl_3$ containing 3.8 mM **2** revealed a $Na^+/2$ ratio of 1.2 ± 0.1 . This indicates that 2 to 3 cations are bound within the β -barrel of dimer 2^2 , which thus may represent an intriguing self-assembled "cation-wire"¹³ capable of spanning the hydrophobic core of EYPC bilayers.

In summary, the above results imply that, in sharp contrast to tetra-Leu **3**, rigid-rod octa-Leu **2** forms stable, mainly (or even exclusively) dimeric rigid-rod β -barrels with ionophoric properties (2^2), capable of mediating efficient ion transport across lipid bilayers. Studies toward larger toroidal rigid-rod suprastructures similar to 2^n are ongoing.

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