Macroscopic, Hierarchical, Two-Dimensional Self-Assembly**

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Herein we demonstrate the operation of a hierarchy of interactions in the two-dimensional self-assembly of hexagonal poly(dimethylsiloxane) (PDMS) plates at a fluid–fluid (perfluorodecalin (PFD)/water) interface.[1–3] The work uses molecular chemistry at surfaces to control menisci and hence interactions: the work has a strong chemical component, but extends from molecular interactions to interactions at surfaces and between materials and then to meso-scale objects. These objects interact through capillarity, that is, by minimizing the surface area of the PFD/H_2O interface.[4] We controlled the faces of the objects that are attracted to one another by patterning them to be hydrophobic or hydrophilic. Lateral capillary interactions can hold millimeter-sized objects through contacts that are sufficiently strong to withstand modest shear.[1–3, 5] The hydrophobic faces have large, positive[6] menisci (the maximum height of the menisci is approximately the height of the faces for the objects used) and these faces are strongly attracted to one another. The hydrophilic faces have small, negative[6] menisci (the maximum height of the menisci is $<40\%$ of the height of the faces) and these faces are weakly attracted to one another.[7] Faces having positive menisci are repelled by faces having negative menisci. We have called the attractive interactions between menisci having the same sense (positive or negative) relative to the interface "capillary bonds", in loose analogy to chemical bonds.[1]

In previous work we assembled objects with flat faces into arrays by means of a single type of interaction. Herein we demonstrate the operation of a hierarchy of capillary interactions between concave and convex faces of different shapes that enables the assembly of complex structures. This "hierarchical self-assembly" illustrates a new type of control in meso-scale self-assembly.

Hierarchical self-assembly is the formation of an ordered structure through a set of interactions that decreases in strength—that is, through a hierarchy of interactions.[8] Hierarchical interactions are ubiquitous in biological systems. The assembly of a viral capsid,[9] for example, involves three steps: a) the synthesis of polypeptide chains through covalent bonds; b) the folding of these polypeptide chains into proteins with well-defined tertiary structures through largely noncovalent intramolecular interactions (van der Waals interactions, hydrogen bonds, and electrostatic interactions); and c) the assembly of these folded proteins into the viral capsid through noncovalent intermolecular interactions (Figure 1 a).

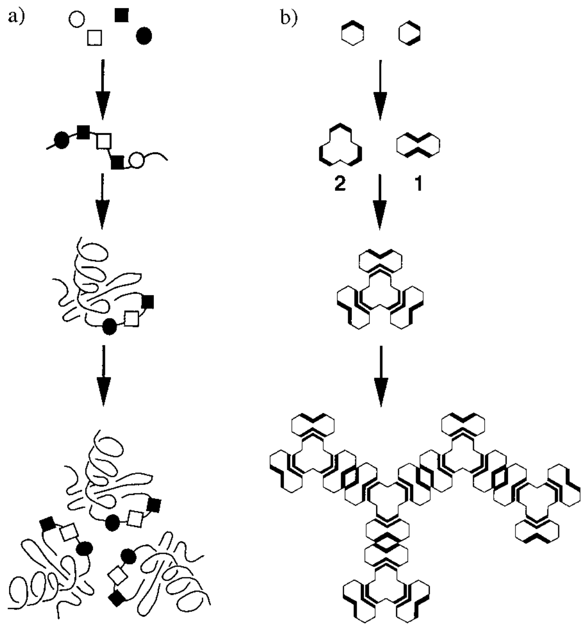

Figure 1. a) Schematic representation of the hierarchical self-assembly of a viral capsid. The squares and circles indicate amino acids that are covalently joined to form a polypeptide. The polypeptide folds into an ordered protein (a form of self-assembly). The proteins aggregate into the viral capsid; b) An example of hierarchical self-assembly of meso-scale polymeric objects. The hexagons represent the hexagonal plates of PDMS used in the experiments, and the thick lines indicate the hydrophobic faces that are involved in the attractive capillary interactions. Appropriately patterned hexagons were glued together to form C_3 (trefoil) or C_2 structures. The C_3 trefoil structure interacts through capillarity with three complementary plates to form an ordered [1 + 3] structure. These [1+3] structures aggregate into an ordered larger structure by interaction between the hydrophobic faces of the C_2 pieces.

Our hierarchy of interactions between the individual components—hexagonal PDMS plates with the faces patterned to be either hydrophobic or hydrophilic—was achieved in three steps (Figure 1 b): a) the strongest connections were permanent, and were formed by gluing hexagonal plates together; b) the strongest capillary interactions occurred between concave and convex hydrophobic faces with complementary shapes and large hydrophobic areas; c) the weakest interactions occurred between two concave hydrophobic faces (Figure 2). In the last two steps a number of different arrays could assemble from the components, so we designed the components to assemble preferentially into only one array. The meso-scale self-assembly illustrated in Figure 1 b is not analogous in any detailed sense to the biological self-assembly sketched in Figure 1 a other than that both involve a hierarchy of interactions, some irreversible and some reversible under the conditions of assembly. Nonetheless, the fundamental concept of "hierarchy" threads through both, and the biological details provide stimuli for future nonbiological studies.

We carried out the experiments that yielded the systems shown schematically in Figure 1 b in three steps. First, we assembled the structures **1** and **2** separately to demonstrate the various arrays that could assemble. Second, we allowed structures **2** and **3** to self-assemble into ordered arrays. Third, we assembled a more complex array from structures **1** and **2**.

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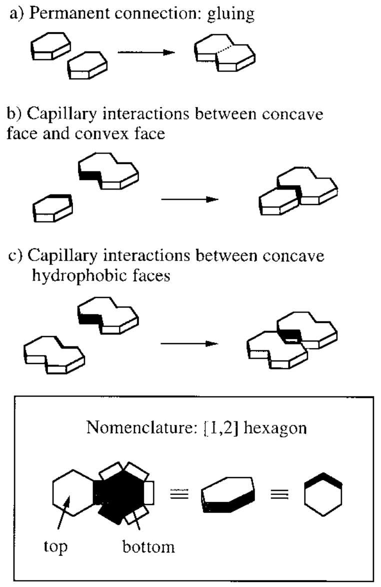

Figure 2. The strength of connections between hexagonal plates can be segregated into an ordered set by using three strategies: a) connecting the plates irreversibly by gluing them together; b) utilizing the capillary interactions between convex and concave faces; c) utilizing the capillary interactions between concave hydrophobic faces. The hexagons used in the work had a face width of 2.7 mm. The thick lines indicate hydrophobic faces and the thin lines indicate hydrophilic faces. In all of the experiments described here the bottom face of the plates was hydrophobic and the top was hydrophilic. The relative disposition of the hydrophobic sides is indicated by [*x*,*y*,..]; thus a [1,2] hexagon is a hexagon with two adjacent sides (and the bottom) hydrophobic, and all other sides (and the top) hydrophilic.

The difference in the arrays that assemble from **1** and **2** separately and the arrays that assemble from a mixture of **1** and **2** demonstrates the control in the hierarchy of capillary-bond strength. We designed **1** and **2** to favor one array among the many that could form.

We fabricated individual structures by using the gluing technique to provide strong, permanent connections. Structure **1** was fabricated by gluing together two [1,3] hexagonal plates,[10] structure **2** by gluing together three [1,2] hexagonal plates, and structure **3** by gluing together two [1] hexagonal plates. The plates were fabricated as follows: PDMS was cured in a hexagonal mold and then removed. The hexagonal rod of PDMS was dyed blue or red by soaking it in either a solution of crystal violet in CH_2Cl_2 or Sudan red B in hexane/EtOH (1/1), respectively.[1] The rod was dried to remove solvent. The faces that were to remain hydrophobic were covered with tape, while others that were to become hydrophilic were left exposed. The rods were cut into pieces and oxidized in a plasma cleaner (Harrick PDC-23G apparatus operating at the medium setting for 5 min). The tape was removed and the plates were cut and placed at the PFD/H_2O interface with tweezers (for a more complete description, see reference [1]). The hexagons, with a thickness of about 1 mm and a diameter of 5.4 mm, were easily manipulated by hand. The two-plane liquid–liquid system (200 mL of PFD and 200 mL of H_2O) was contained in a Petri dish 14.5 cm in diameter, and swirled on an orbital shaker at a frequency ω between 0.6 and 1.8 s^{-1}. The assemblies were complete in 3–5 h. We performed each experiment at least five times and in many cases more.

Figure 3a and b show the arrays that formed when objects **1** and **2** were allowed to self-assemble separately. We agitated the objects at $\omega = 0.6\,s^{-1}$ (Figure 3a) or $\omega = 1.5\,s^{-1}$ (Figure 3b). In these assemblies we used 1.4-mm-thick objects of **1** and **2**. These optical micrographs establish that self-assembly yields ordered arrays, but give no indication of the relative strength of the two interactions that hold each aggregate together. We believe that the interaction between the plates in Figure 3a is weaker than that in Figure 3b since the aggregate $\mathbf{1}_n$ broke apart under milder agitation ($\omega > 0.6\,s^{-1}$) than $\mathbf{2}_n$ ($\omega > 1.5\,s^{-1}$).

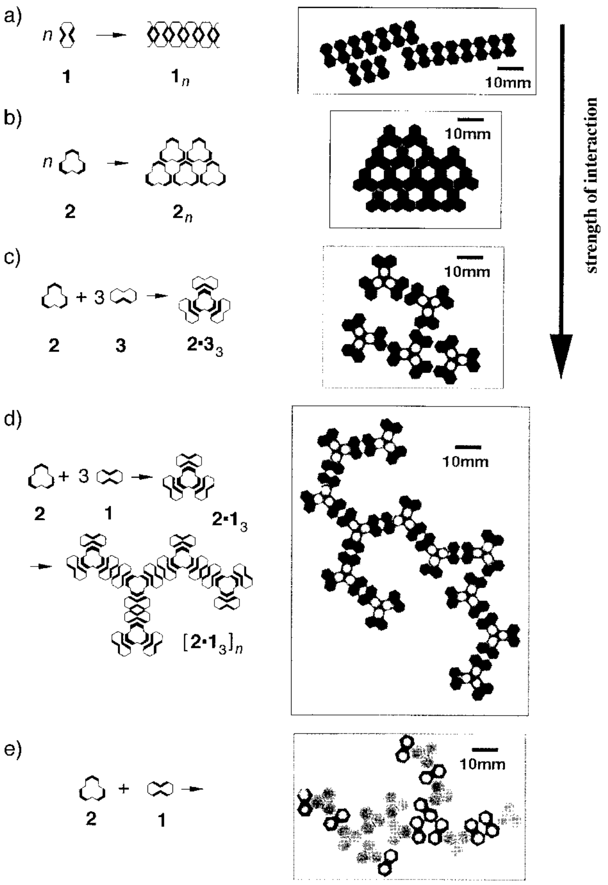

Figure 3. a) Formation of an extended linear array $\mathbf{1}_n$ from **1** by interactions of menisci at concave hydrophobic sides. The optical micrograph also suggests weak attractive interactions between hydrophilic sides. In this system, where the density of PDMS is approximately equal to the density of H_2O, the menisci of water at hydrophilic sides (negative menisci) are small, and capillary interactions between these menisci are substantially weaker than those between menisci of PFD at hydrophobic sides; b) Self-assembled structure $\mathbf{2}_n$ based on the oligomerization of hydrophobic faces of **2**; c) Structure $\mathbf{2}\cdot\mathbf{3}_3$ assembled from three objects of **3** and one of **2**. The further interaction of these structures through menisci at the hydrophilic faces was weak. Structure **3** was dyed a uniform color and structure **2** was dyed with three clear circles in the center of each hexagon; d) An extended hierarchical array $[\mathbf{2}\cdot\mathbf{1}_3]_n$ of twelve [1 + 3] aggregates derived from **1** and **2**; e) Disordered, self-assembled aggregate formed from a 1:1 mixture of **1** and **2**.

To demonstrate the level of control over the final assembly that can be designed into this system, we allowed **2** and **3** to self-assemble (Figure 3c). Here we used 0.9-mm-thick objects of **2** and 1.4-mm-thick objects of **3**.[11] The frequency of agitation was periodically changed between 1.5 and 1.8 s^{-1}.[12] A variety of structures could, in principle, form on self-assembly: $\mathbf{2}_n$, $\mathbf{3}\cdot\mathbf{3}$, $\mathbf{2}\cdot\mathbf{3}_1$, $\mathbf{2}\cdot\mathbf{3}_2$, and $\mathbf{2}\cdot\mathbf{3}_3$. If the interactions between **2** and **3** were equal in strength we would expect a nearly statistical distribution of these structures. In fact, the structure $\mathbf{2}\cdot\mathbf{3}_3$ formed in approximately 70% yield.[13] We designed the interaction between **2** and **3** to be the strongest interaction, and this interaction was observed most frequently. The interaction between **3** and **3** was weaker than that between **2** and **3** because the two concave faces of **3** were not complementary in shape.

We also examined the influence of the thickness of **3** on the self-assembly of **2** and **3** (Figure 4). We demonstrated previously that the capillary interactions between thick, flat objects are stronger than those between thin, flat objects of the same lateral dimensions.[3] The assembly with 0.9-mm-thick objects of **3** gave a yield similar to that with 1.4-mm-thick **3**. When we allowed **2** and 2.0-mm-thick objects of **3** to assemble we obtained the desired structure $\mathbf{2}\cdot\mathbf{3}_3$ in a lower yield (40%). In this case, we believe that we had a lower yield because the interaction between the concave faces in $\mathbf{3}\cdot\mathbf{3}$ was strong.[14] We conclude that the height of **3** made only a small contribution to the formation of the assembly.

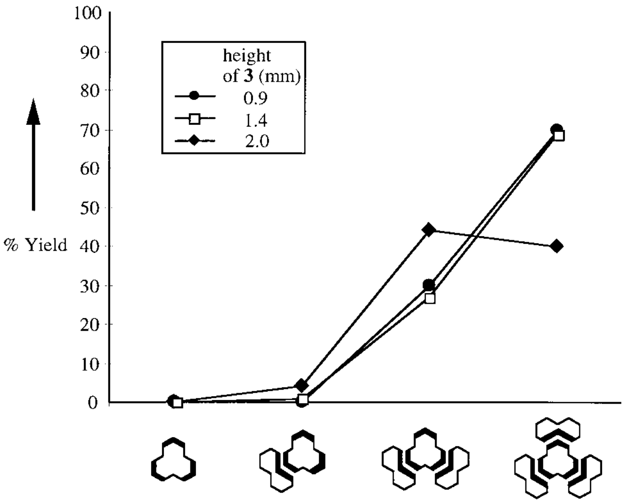

Figure 4. The yield for each of the possible arrays that assembled from **2** and **3** as a function of the height of **3** (for a constant height of **2** of 0.9 mm). The inset shows the symbols corresponding to the various thicknesses of **3**. We calculated the yields based on **2**. We repeated the experiment five or six times with six objects of **2** and eighteen of **3**. We agitated the objects for 1 h. The graph indicates that going to a thickness of **3** of less than 0.9 mm may result in a higher yield for $\mathbf{2}\cdot\mathbf{3}_3$. We were limited by the method of fabrication and could not fabricate plates thinner than 0.9 mm.

To build more complex structures we introduced a further weak interaction: the interaction between menisci at concave faces (Figure 2c). We first allowed 36 objects of **1** (1.8-mm thick) and 12 of **2** (0.9-mm thick) to assemble at a rotation frequency of 1.5 s^{-1} (the rate of agitation was periodically cycled between 1.5 and 1.8 s^{-1} to accelerate annealing and to break up weakly associated structures) into the structure $\mathbf{2}\cdot\mathbf{1}_3$ (Figure 3d).[15] The strongest capillary interaction between complementary hydrophobic faces caused assembly of $\mathbf{2}\cdot\mathbf{1}_3$. A weaker capillary interaction between two concave hydrophobic faces enabled $\mathbf{2}\cdot\mathbf{1}_3$ to assemble into larger aggregates, when the agitation was decreased to a frequency of 0.6 s^{-1}. We did not observe a cyclic array of $[\mathbf{2}\cdot\mathbf{1}_3]_n$ under any conditions.

Figure 3e shows the aggregate that forms on self-assembly of a 1:1 mixture of **1** and **2**. These objects, in this stoichiometry, could, in principle, assemble into a hexagonal lattice, but we did not observe this array experimentally. The aggregates consist of a partially assembled structure of **1** and **2** ($\mathbf{2}\cdot\mathbf{1}_2$ and $\mathbf{2}\cdot\mathbf{1}_1$) and $\mathbf{2}_n$. Aggregates of **2** as well as $\mathbf{2}\cdot\mathbf{1}_2$ and $\mathbf{2}\cdot\mathbf{1}_1$ (and sometimes $\mathbf{2}\cdot\mathbf{1}_3$) were observed under agitation. We could not adjust the agitation in a way that generated a hexagonal lattice.

This demonstration illustrates the operation of a hierarchy of interactions of different strengths. The strategy of hierarchical self-assembly is one that was motivated by systems of biological molecules, but exists also in many other systems.[16, 17] Although these initial assemblies are far from biological systems in terms of complexity, we believe that their formation, and the principles of design on which they are based, point the way to new strategies for assembling complex meso-scale aggregates.

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[1] N. Bowden, I. S. Choi, B. A. Grzybowski, G. M. Whitesides, *J. Am. Chem. Soc.* **1999**, *121*, 5373–5391.

[2] I. S. Choi, N. Bowden, G. M. Whitesides, *J. Am. Chem. Soc.* **1999**, *121*, 1754–1755.

[3] N. Bowden, A. Terfort, J. Carbeck, G. M. Whitesides, *Science* **1997**, *276*, 233–235.

[4] There is also a gravitational term in the capillary forces, which results from the lowering of the liquid in the menisci to the level of the interface. The gravitational term is, in general, smaller than the energy associated with the change in the surface area.

[5] a) V. N. Paunov, P. A. Kralchevsky, N. D. Denkov, K. Nagayama, *J. Colloid Interface Sci.* **1993**, *157*, 100–112; b) P. A. Kralchevsky, V. N. Paunov, N. D. Denkov, I. B. Ivanov, K. Nagayama, *J. Colloid Interface Sci.* **1993**, *155*, 420–437.

[6] We call the menisci on the hydrophobic faces that form when PFD wets these faces "positive" menisci (menisci rising above the *x,y* plane of the interface) and those on the hydrophilic faces that form when H_2O wets these faces "negative" menisci (menisci sinking below the *x,y* plane of the interface).

[7] Objects with an asymmetric pattern of hydrophobic faces tend to tilt and raise a hydrophilic face above the PFD/H_2O interface. Small, positive menisci form on the hydrophilic faces tilted out of the interface as a consequence of the PFD that wets the bottom, hydrophobic face. These positive menisci are often small and only weakly attracted to other positive menisci.

[8] "Hierarchy" is defined in *The American Heritage College Dictionary*, 3rd ed., as a series in which each element is graded or ranked.

[9] a) D. Chang, C.-Y. Fung, W.-C. Ou, P.-C. Chao, S.-Y. Li, M. Wang, Y.-L. Huang, T.-Y. Tzeng, R.-T. Tsai, *J. Gen. Virol.* **1997**, *78*, 1435–1439; b) W. Yang, J. Guo, Z. Ying, S. Hua, W. Dong, H. Chen, *J. Virol.* **1994**, *68*, 338–345; c) S. B. Larson, S. Koszelak, J. Day, A. Greenwood, J. A. Dodds, A. McPherson, *Nature* **1993**, *361*, 179–182; d) R. C. Liddington, Y. Yan, J. Moulai, R. Sahli, T. L. Benjamin, S. C. Harrison, *Nature* **1991**, *354*, 278–284; e) F. Birnbaum, M. Nassal, *J. Virol.* **1990**, *64*, 3319–3330.

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[10] For the nomenclature used to describe hexagonal plates, see Figure 2 and Reference [1].

[11] The thickness of the plates was controlled by cutting the oxidized hexagonal rod with a razor blade. The thickness is an average value since the hexagons vary slightly in their thickness (±0.1 mm).

[12] We periodically changed the rate of agitation to 1.8 s^{-1} to accelerate collisions, to break up weak structures, and to stimulate annealing.

[13] We repeated the experiment six times. The yield was calculated by counting the number of times structure $\mathbf{2}\cdot\mathbf{3}_3$ was formed, relative to the incomplete structures $\mathbf{2}\cdot\mathbf{3}_2$ and $\mathbf{2}\cdot\mathbf{3}_1$. For example, in the assembly of 1.4-mm-thick objects of **3** and 0.9-mm-thick **2** we counted 21 arrays of $\mathbf{2}\cdot\mathbf{3}_3$, 3 arrays of $\mathbf{2}\cdot\mathbf{3}_2$, and 6 arrays of $\mathbf{2}\cdot\mathbf{3}_1$. The yield of $\mathbf{2}\cdot\mathbf{3}_3$ was 21/30 or 70%.

[14] The structure $\mathbf{2}\cdot\mathbf{3}_3$ was not stable at $\omega = 1.5\ s^{-1}$, but was stable at $\omega = 1.2\ s^{-1}$. We agitated the objects at $\omega = 1.2\ s^{-1}$ and periodically changed the frequency of agitation to 1.8 s^{-1}.

[15] We repeated the experiment five times with six objects of **2** and eighteen of **1** to get statistics for this self-assembly. We agitated the plates for one hour. The yields of the assembly of $\mathbf{2}\cdot\mathbf{1}_3$ were 67%, 100%, 83%, 67%, and 67%. The micrograph shown in Figure 3d is the most complete assembly (the highest yield) that we observed.

[16] S. A. Jenekhe, X. L. Chen, *Science* **1999**, *283*, 372–375.

[17] D. E. Ingber, *Sci. Am.* **1998**, *278*(1), 48–57.

Stereocontrolled Synthesis of (−)-5,11-Dideoxytetrodotoxin**

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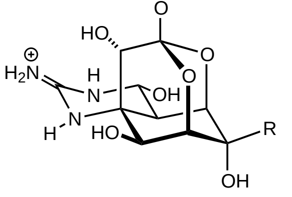
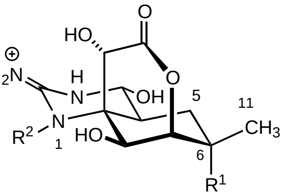
Tetrodotoxin (**1**)[1] is a well-known marine natural product that is the toxic principle of puffer fish poisoning. Due to its highly selective inhibition of voltage-dependent sodium channels, tetrodotoxin has served widely as an important biochemical tool in neurophysiological studies.[2] Recently, tetrodotoxin analogues have been isolated from many kinds of animals.[3] These studies brought to light a number of new interesting problems associated with tetrodotoxin,[4] for example its biosynthesis, mechanisms of accumulation, detoxification, and binding to the sodium channel protein,[5] and its actual biological function.[6] In order to examine such new problems on a molecular level, suitably labeled tetrodotoxins are desirable. Such labeled compounds, however, are difficult to prepare from naturally occurring tetrodotoxin.[7] The complex structure, which has a variety of functional groups and unique chemical properties, has thwarted recent attempts at its total synthesis. In spite of many efforts,[8] no total synthesis of tetrodotoxin has been reported since the total synthesis of the racemate by Kishi, Goto, and co-workers in 1972.[9] During the course of our synthetic studies on tetrodotoxin, we have developed a stereocontrolled synthesis of polyhydroxylated cyclohexane,[10] the introduction of a nitrogen functionality by the Overman rearrangement,[11, 12] and a novel guanidine synthesis.[13] Here we describe the stereocontrolled synthesis of enantiomerically pure 5,11-dideoxytetrodotoxin (**2**), a tetrodotoxin analogue not yet found in natural sources.

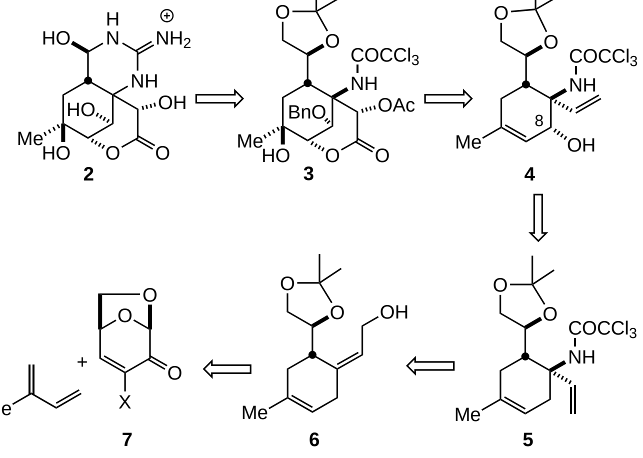


Tetrodotoxin **1** R = CH_2OH
11-Deoxytetrodotoxin R = CH_3



5,6,11-Trideoxytetrodotoxin $R^1 = H$ $R^2 = H$
1-Hydroxy- 5,11-Dideoxytetrodotoxin $R^1 = OH$ $R^2 = OH$
5,11-Dideoxytetrodotoxin **2** $R^1 = OH$ $R^2 = H$

Our retrosynthetic plan for 5,11-dideoxytetrodotoxin (**2**) is shown in Scheme 1. The guanidine part of **2** is introduced at the latest stage of the synthesis because of the instability and low solubility of the intermediates. Lactone intermediate **3** was envisioned to be synthesized from trichloroacetamide diene **5** via diene alcohol **4** in a stereoselective manner. Compound **5** has already been synthesized from levoglucosenone **7** (X = H) as a chiral starting material[14] by means of a



Scheme 1. Retrosynthetic plan for 5,11-dideoxytetrodotoxin (**2**).

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