pected composition and spatial arrangement of a self-assembled monolayer (e.g., average polymethylene chain tilt of ~30° from the surface normal[8b-d]). Though not as extensively examined, we have also electrodeposited monolayers of other thiolates (i.e., n = 1–15 for X = CH_3, n = 2 for X = $CF_3(CF_2)_7$, n = 1, 2 for X = COOH, and n = 2, 3 for X = OH), demonstrating that the electrodeposition process is, indeed, a general route for the formation of thiolate monolayers.

Studies to characterize further the thermodyamics of the adsorption/desorption process are underway. We are also beginning to explore the application of electrodeposition for site-selective monolayer formation on multielement electrode arrays and for the formation of partial and mixed monolayers of known composition.

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Chemically-Induced Aggregation, Budding, and Fusion in Giant Vesicles: Direct Observation by Light Microscopy

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Albert Einstein once wrote that "the only apparatus necessary for observing Brownian motion is the microscope, and it need not even be a particularly good one".[1] A similar sentiment can be expressed for the topic of this paper, membrane motion. We describe herein a synthetic system[2] that undergoes a remarkable set of morphological events, all of them chemically-induced and all of them visible under the microscope.[3] These include aggregation, budding, and fusion.

Direct microscopic observation of membrane mechanics in biomimetic systems was made possible by Reeves and Dowben,[4] who first described the synthesis of giant vesicles in which a single bilayer surrounds a space as large as a living cell. In our experiments, we used $(C_{12}H_{25})_2N^+(CH_3)_2Br^-$, a cationic amphiphile (T_c = 17 °C) henceforth called DDAB. Giant vesicles (10–200 μm in diameter) were formed by soaking a thin DDAB film in 50 °C water for about an hour and then shaking for 4 s. Membrane properties were observed with a microscope (a good one as it happened): a Leitz Laborlux S equipped with phase-contrast and dark-field illumination.

Giant vesicles (200 μL of 10 mg DDAB/20 mL water) were placed within the confines of a washer cemented onto a microscopic slide. To begin an experiment, 100 μL of an additive was injected into the center of the vesicle solution, and the mixture was examined at a 320× magnification (Figure 1).

Figure 1A shows that low levels of Na_2SO_4 (1.0 mM) cause DDAB giant vesicles, initially isolated from one another, to aggregate over several minutes. No fusion occurs (as had been previously reported[5] for sulfate added to submicroscopic vesicles

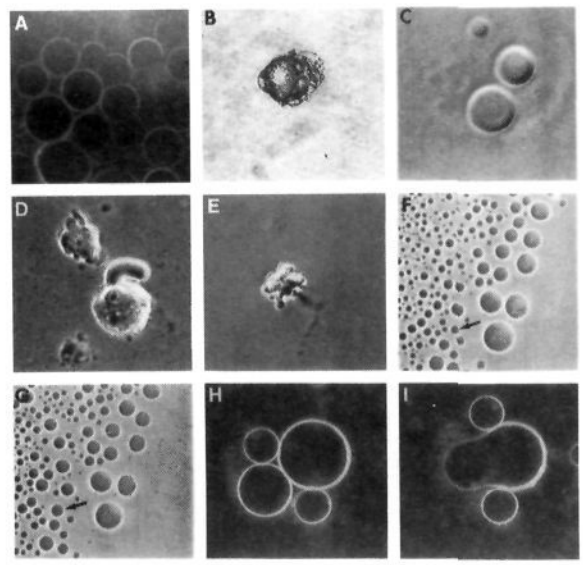

Figure 1. A: Aggregation of DDAB vesicles induced by sodium sulfate. Initially, only 3–4 vesicles were present in the microscopic field. B: Coagulation induced by sodium bromide. C: Flattening induced by equiosmolar glucose. D: Budding and vesicle ejection induced by sodium acetate. E: Continuation of the process seen in D in which all vesicles disappear to produce a clear microscopic field. F and G: Reappearance of vesicles after a few minutes. New vesicles fuse rapidly (compare vesicles indicated by arrows; photos F and G were taken less than 1 min apart). H and I: Dark-field photo of two fusing vesicles. Vesicles can sit side-by-side for many minutes, but fusion, once it begins, takes only seconds. Fused vesicles always reestablish a spherical shape (not shown). Experiments were carried out at 5–6 deg above the phase transition of the synthetic lipid. Additives were injected at a concentration of 0.1–0.3 M, except for the sodium sulfate which was used at a 1 mM concentration to avoid precipitation.

80 nm in diameter). High curvature clearly promotes the uniting of membranes. According to current dogma,[6] aggregation in the absence of fusion signifies that sulfate can neutralize long-range double-layer repulsion. On the other hand, sulfate apparently cannot subdue short-range "hydration repulsion" as would be necessary for fusion. Hydration forces reflect the need to desolvate headgroups prior to phase instability in the membrane.[7]

Injection of 0.1 M NaBr into the giant vesicle sample causes the DDAB to precipitate into a solid mass (Figure 1B). This is not purely an osmotic effect because equiosmolar glucose manages only to flatten the vesicles at the poles (Figure 1C). Salt-induced coagulation in submicroscopic vesicles is a well-studied phenomenon.[8]

Addition of NaOAc (0.1–0.3 M) leads to two striking effects: (a) the giant vesicles eject smaller vesicles by a budding process (Figure 1D,E). These small vesicles ultimately diminish in size and disappear to create a microscopically clear field. (b) After about 15 min, however, the field becomes speckled with newly formed vesicles that fuse at a prodigious rate (Figure 1F,G). The dark-field photos in Figure 1H,I catch two vesicles in the coupling act.

The acetate effect can be understood in terms of two notions: (a) strongly hydrated anions, such as acetate, bind relatively loosely to cationic surfaces,[9,10] and (b) according to the Svetina–Zeks model,[11] the coupled leaflets in a bilayer can act independently.

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Thus, when excess acetate is added externally to the DDAB vesicles, acetate exchanges with bromide to produce an outer leaflet that is more highly dissociated from its counterions. Owing to the resulting headgroup–headgroup repulsion, the outer leaflet expands relative to the inner one. Such asymmetry would be expected to increase curvature[12] and, therefore, promote budding and expulsion of small vesicles, as was observed.

The submicroscopic vesicles (and/or micelles) reassemble into giant vesicles, this time with acetate more equally distributed on the two sides of the vesicle walls. The question then arises as to why subsequent fusion is so fast in the face of what certainly must be a severe electrostatic barrier between positively charged membranes. There are two possible answers: (a) hydration repulsion may, for unknown reasons, not manifest itself in our particular system. This seems unlikely because charged bilayer surfaces should, if anything, require particularly strong solvation. (b) The vesicles may fuse because the component amphiphilic molecules are loosely packed within the bilayer assembly. This latter possibility receives support from additional observations: the dioctadecyl analog of DDAB, with T_c = 37 °C, does not exhibit acetate-induced fusion at ambient temperatures where the bilayer exists in the rigid gel state.[13] Moreover, DDAB vesicles, stiffened with 20 mol % cholesterol, fuse very slowly upon acetate injection.

Membrane–membrane interactions are usually analyzed in terms of three additive forces: van der Waals attraction, electrostatic repulsion, and hydration repulsion. Our results suggest that a fourth energy term, a "packing" or "stiffness" factor, must also be taken into account at the very close proximities required for fusion.[14] Indeed, fusion kinetics may be controlled more by the energetics of reassembling and mixing organized molecules[15] than by the need to overcome hydration forces.

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New 4 + 1 Radical Annulations. A Formal Total Synthesis of (±)-Camptothecin

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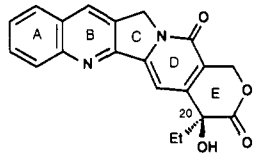
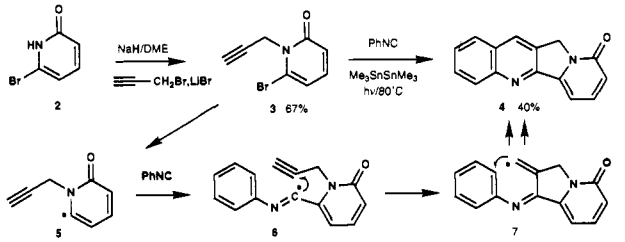
Over the last decade, (20*S*)-camptothecin (**1**)[2] and its close relatives have emerged as some of the most exciting compounds for potential treatment of solid tumors.[3] Very recently, camptothecin has also shown potent anti-retroviral activity at dose levels well tolerated by cells.[4] It may therefore represent a new direction in AIDS chemotherapy. Derivatives of camptothecin have a unique mechanism of action: they kill cells by binding to and stabilizing a complex of DNA and the enzyme topoisomerase I.[5]



camptothecin (1)

These compounds are the most important members of a very small group of compounds[6] known as "topoisomerase I poisons".[7] Camptothecin was synthesized several times during the 1970s,[2,8] and many (though not all) syntheses rely on the Friedlander quinoline synthesis to construct ring B. Given the current interest in camptothecins, new directions in the total synthesis of this family would be welcome. We now report a short, convergent total synthesis of (±)-camptothecin that uses a new 4 + 1 radical annulation[9a] followed by another cyclization[9] to simultaneously assemble rings B and C.

The viability of the key 4 + 1 annulation was first demonstrated in a simple model reaction. Readily available bromopyridone **2**[10] was N-propargylated to give **3** (eq 1). In turn, **3** reacted with phenyl isocyanide under conditions similar to those that we developed for reactions of simple pentynyl iodides.[9a] An 80 °C benzene solution of **3** (1 equiv), phenyl isocyanide (5 equiv), and hexamethylditin (1.5 equiv) was irradiated with a sunlamp for 8 h. After chromatography, we isolated the known tetracycle **4**[11] in 40% yield as a white solid. Equation 1 shows key steps in the proposed mechanism for the conversion of **3** to **4**. Addition of pyridone radical **5** to phenyl isocyanide[12] to give **6** is followed by two radical cyclizations and an oxidative rearomatization.[9,13]



(1)

The formal total synthesis of (±)-camptothecin is shown in eq 2. Nitrile **8** was prepared by standard Doebner condensation of dimethyl acetonedicarboxylate and cyanoacetic acid (70%).[14] Standard saponification (NaOH/EtOH, 95%) gave diacid **9**. Conversion of **9** to bromopyridone **10** was accomplished by modification of a known method to prepare chloropyridones.[14] The

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