Table II. Effect of Solvents on Transition-State Geometries and Dipole Moments

X	conf	ε	r(C=O)	NC···C	∠NC···C=O	μ
CH_3	anti	1.0	1.2386	1.9364	113.39	6.91
	anti	78.5	1.2532	1.8411	111.12	7.75
	+sc	1.0	1.2402	1.9188	112.44	6.47
	+sc	78.5	1.2512	1.8691	110.16	7.65
	-sc	1.0	1.2415	1.9142	113.00	5.72
	-sc	78.5	1.2515	1.8531	111.28	6.62
F	anti	1.0	1.2362	1.9055	113.94	5.00
	anti	78.5	1.2457	1.8575	111.80	5.83
	+sc	1.0	1.2302	2.0095	113.06	6.09
	+sc	78.5	1.2433	1.9091	111.16	6.70
	-sc	1.0	1.2276	1.9837	113.98	6.17
	-sc	78.5	1.2406	1.8959	111.34	6.94
SiH_3	anti	1.0	1.2358	1.9824	113.37	7.84
	anti	78.5	1.2526	1.8572	110.88	9.07
	+sc	1.0	1.2394	1.8933	112.61	6.79
	+sc	78.5	1.2494	1.8616	110.05	8.67
	-sc	1.0	1.2391	1.9640	113.25	7.42
	-sc	78.5	1.2491	1.8847	111.60	7.84
CN	anti	1.0	1.2255	1.9673	114.07	4.39
	anti	78.5	1.2325	1.9271	112.21	5.31
	+sc	1.0	1.2230	2.0200	112.64	5.94
	+sc	78.5	1.2338	1.9206	111.21	6.49
	-sc	1.0	1.2225	1.9959	114.54	6.54
	-sc	78.5	1.2333	1.9191	112.00	7.42

are given in Table I. It can be seen that the preferred conformer is solvent dependent. When X = CH_3, the -sc conformer is predicted to be preferred in the gas phase, and it has the lowest dipole moment (5.72 D). If everything else were equal, one would expect the conformer with the lowest dipole moment to be favored in the gas phase. In solution, the anti conformer is predicted to be more stable. With X = SiH_3, the -sc form is favored in the gas phase, as was found with X = CH_3. Here, the +sc form has the lower dipole moment, but the -sc form would have a favorable attractive interaction between the positively charged silicon and the negatively charged oxygen. In solution, this would be less important, and now the anti form is favored. With X = F, the anti form with the lower dipole moment is favored in the gas phase, but the +sc form is favored in solution. Finally, with X = CN, the anti form with the lowest dipole moment is again favored in the gas phase, but now all conformers have comparable energies in solution. It appears that the conformational preferences for the gas phase are largely determined by electrostatic interactions which are minimized on going to a solvent.

It is, of course, possible that the change in conformer preference might reflect a change in the ground-state conformational energies of the reactant aldehydes on going from the gas phase to solution.[4a] This was examined in the case of fluoroacetaldehyde. The +sc transition state corresponds to the 180° (anti) ground-state conformer, whereas the anti and -sc transition states correspond to the 70° (gauche) rotational transition state for fluoroacetaldehyde. The anti → gauche energy difference for the aldehyde is calculated (RHF/6-31G*) to be 5.7 kcal/mol in the gas phase, and it decreases to 3.5 kcal/mol in solution. Thus, if the change in ground-state conformer energy difference applied to the cyanide addition transition states, the anti TS should drop in energy by 2.2 kcal/mol relative to +sc on going from the gas phase to solution. However, the contrary is true, with the anti TS going up in energy by 1.6 kcal/mol relative to +sc.

Some key structural parameters for the transition states are recorded in Table II. The effect of solvents is to increase the C=O bond length, decrease the NC···C distance, decrease the angle at which the CN^- attacks the carbonyl, and increase the dipole moment. Therefore, the reaction appears to have progressed further toward products in solution than in the gas phase.

These data indicate the importance of considering solvent effects in determining π-facial selectivity. The origin of the conformational preferences in solution will be considered in detail in a full paper describing this work.

Calculations. The calculations were carried out using GAUSSIAN-92.[9] The SCRF calculations need a cavity size. It was chosen in each case using a molecular volume derived from the 0.001 e/B^3 surface[10] plus an empirically derived value of 0.5 Å to account for the nearest approach of solvent molecules.[7a] The cavity radii used were the following: X = CH_3, 3.91 Å; X = F, 3.70 Å; X = SiH_3, 4.09 Å; X = CN, 3.93 Å.

Acknowledgment. This investigation was supported by a grant from the National Institutes of Health.

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Bilayer-Bridging Bolaamphiphilic Lipids

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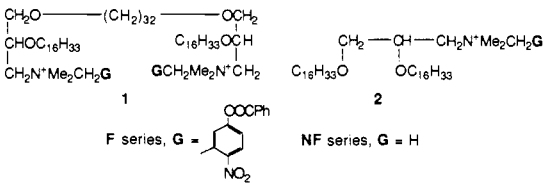
The unusual bolaamphiphilic phytanyl lipids isolated from the thermophilic (50–80 °C) archaebacteria have been the subjects of structural, modeling, and synthetic studies.[1,2] Understanding the relation between their molecular structure and their function within the bacterial membranes could lead to "designer" lipids and liposomes of enhanced thermal stability. The 32-carbon main chain of such lipids permits them to bridge typical membranes, anchoring their head groups at both outer and inner aqueous/lipid interfaces.[1a] To explore whether this affords enhanced membrane stability, model lipids were synthesized.[1a,c,e,2b] Evidence from permeability[1c] and structural studies[1a,e] suggests greater robustness for liposomes bolstered by bridging bolaform lipids.

Our interest in lipid molecular structure and intraliposomal dynamics[2,3] led us to prepare the desmethylated, model functional bolaamphiphile ("bola") **1-F**.[2b] We dispersed **1-F** in conventional bilayer liposomes of the nonfunctional, monopolar host lipid **2-NF**,[2b] surface specifically cleaved[3c] the outer ester head groups of **1-F**, and followed the dynamics of **1-F** lipid reequilibration in the resultant "differentiated" coliposomes.[2b] At least 40% of the



1-F molecules adopted nonbridging, U-plan arrangements in the outer leaflet of the **2-NF** bilayer (Figure 1, pattern x), and the observed inner → outer, transbilayer ("flip-flop") dynamics of the inner liposomal head groups of **1-F** were very similar to those of simple monopolar functional lipids in bilayers of **2-NF**.[2b] There was no evidence for extensive bridging by **1-F** or for enhanced thermal stability in these **1-F**/**2-NF** coliposomes.

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Table I. Properties of Coliposomes

coliposome[a] (composition)	d (nm)[b]	T_c (°C)	k_f (s^{-1})[c]	k_s (s^{-1})[c]	phasing[d]	$t_{1/2}$ flip[e]
2-F/2-NF[f] (1:7)	48	37	0.075[g]	0.0010[g]	67:33	5 min/40 °C; 1 min/45 °C
5-F/5-NF (1:7)	38	31	0.056[g]	0.000060[g]	60:40	>60 min/30 °C; 2 min/40 °C
1-F/2-NF[h] (1:14)	30	39	0.14[g]	0.00058[g]	70:30	5 min/35 °C; 2 min/40 °C
3-F/5-NF (1:28)	34	34	0.040[i]	0.000026[j]	50:50	>60 min/60 °C[e]
4-F/5-NF (1:28)	37	*k*	0.035[i]	0.000045[j]	50:50	>60 min/60 °C[e]

[a] See text for structures and conditions. [b] Diameters from dynamic light scattering at pH 4, 0.01 M KCl. [c] k_f and k_s are the rate constants for fast (outer surface) and slow (inner surface) esterolysis of the functionalized lipids. [d] % fast cleavage/% slow cleavage. [e] See text for discussion. Reproducibility, ±1 min. [f] Reference 3b. [g] At 25 °C. [h] Reference 2b. [i] At 15 °C. [j] At 20 °C. [k] Not measured, but assumed to be ~34 °C as found for the 1:28 **3-F/5-NF** coliposomes.

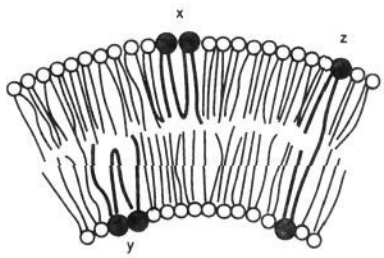
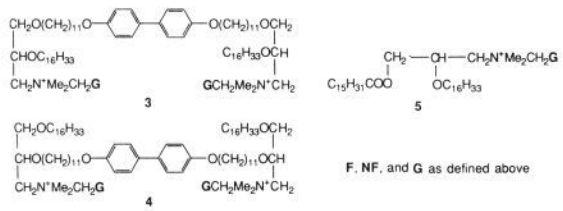


Figure 1. Schematic diagram of possible bolaamphiphile arrangements (dark head groups and bold chains) in a host bilayer of conventional lipid molecules (light head groups). The depicted bola "patterns" are the following: U-plan in the outer (x) or inner (y) leaflets and transbilayer or "bridging" (z).

Now we describe two new, isomeric, biphenyl-stiffened functional bolas, **3-F** and **4-F**. In host bilayer liposomes of **5-NF**, they exhibit transmembrane bridging and unprecedented resistance to thermally driven flip-flop. Syntheses of the new bolas are described in the supplementary material.[4] Coliposomes **3-F/5-NF**[5] and **4-F/5-NF**[5] were generated by sonication of $CHCl_3$-cast films of 1:28 (molar ratio) F/NF surfactant blends at pH 3.9 and filtration through a 0.8 μM Millex membrane.[6] Hydrodynamic diameters, obtained by dynamic light scattering,[3c] were consistent with unilamellar bilayer liposomes[7] (cf. Table I). Gel to liquid crystal transition temperatures (T_c) were determined from the temperature dependence of the fluorescence polarization of included 1,6-diphenyl-1,3,5-hexatriene[8] and are collected in Table I.



Coliposomes prepared at pH 3.9 (HCl, 0.01 M KCl) were surface-differentiated by exposure to 1×10^{-4} M glutathione in 5×10^{-3} M pH 8 Tris buffer, $\mu = 0.01$ M (KCl).[3b,c] *Exoliposomal* *p*-nitrophenyl benzoate esters of the functional (F) lipids were rapidly cleaved (k_f, Table I), affording *p*-nitrophenylate residues monitored spectrophotometrically at 400 nm. *Endoliposomal* esterolysis (k_s) occurred slowly, limited by cation (Na^+, K^+) permeation across the liposomal membranes, driven by the imposed 8:4 exo/endo pH gradient.[3a,9]

The time course of exo and endo esterolyses for the **4-F/5-NF** coliposome is illustrated in Figure 2; the behavior of **3-F/5-NF** (not shown) is analogous. These surface differentiations both proceed with 50% (±2%) of fast, exoliposomal esterolysis and 50%

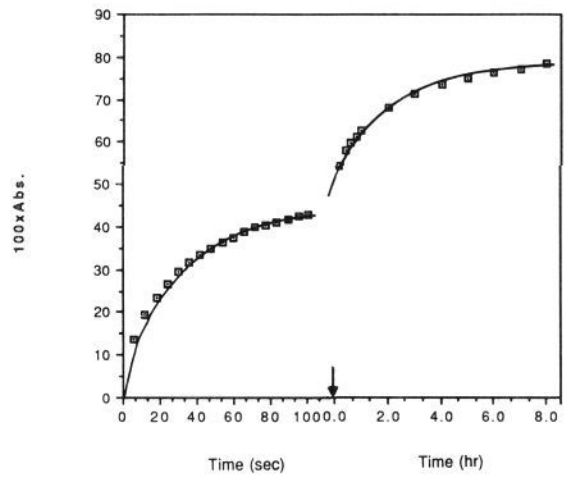


Figure 2. Absorbance at 400 nm vs time for the cleavage of *p*-nitrophenyl benzoate and the appearance of *p*-nitrophenylate head groups from **4-F/5-NF** coliposomes (see Table I and text). Note the discontinuous time axis. A^∞ for the slow reaction was 86 after 24 h.

of slow, endoliposomal reaction, consistent with the demands of bilayer bridging (Figure 1, pattern z).[10]

These 50:50 phasings differ from the 70:30 partition observed with **1-F/2-NF** coliposomes where, due to U-plan bending of **1-F** (Figure 1, pattern x), there is at least a 40% excess of exo functional groups.[2b] Comparable situations are routinely seen with *monopolar* functional lipids in conventional bilayer coliposomes (cf. **2-F/2-NF** and **5-F/5-NF** in Table I). Exoliposomal excesses of functional head groups are expected because these larger head groups should be "concentrated" in the outer leaflet of the **NF** host bilayer, where the curvature is gentler than at the inner surface.[2b]

In principle, the 50:50 phasings of **3-F** or **4-F** in **5-NF** coliposomes are also consistent with substantial numbers of U-plan bolas, *equally* distributed between the inner and outer bilayer leaflets (Figure 1, patterns x *and* y).[11] That is *not* the case as is strongly implied by the unusual dynamic behavior of the differentiated **3-F/5-NF** and **4-F/5-NF** coliposomes.

We detailed an experimental protocol to determine the reequilibration dynamics of exo/endo-differentiated functional lipids.[3,12] Approximate half-times for transverse bilayer (flip-flop) reequilibrations of various functional lipids, thus obtained, appear in Table I. The biphenyl-stiffened bolas, **3-F** and **4-F**, differ from the unstiffened bola, **1-F**, and from monopolar lipids, **2-F** and **5-F**, in that they exhibit far greater resistance to thermally driven flip-flop. Indeed, heating differentiated **3-F/5-NF** or **4-F/5-NF** coliposomes to 40 °C for 1 h induces only 7–8% transfer of their

(4) Appropriate NMR and elemental analytical data were obtained.
(5) Lipid **5-NF** was employed as host lipid because optically clear coliposome solutions of **3-F** or **4-F** could not be obtained with host **2-NF**.
(6) The final concentration of the host (NF) lipid was 3.5×10^{-4} M. The procedure described here generally provides unilamellar liposomes.[3c]
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(10) Exo/endo "phasing" (cf. Table I) is apparent from the ratio of absorbances corresponding to k_f and k_s, respectively. A^∞_{endo} was reached after 24 (**4-F**) or 42 h (**3-F**). Alternatively, after completion of the k_f process, A^∞_{endo} could be quickly determined by heating the coliposomes (75 °C/20 min), where the final absorbance was identical to A^∞_{endo} obtained by continuous monitoring.
(11) This distribution is inherently unlikely due to the effects of curvature.
(12) Differentiated bolas (Figure 1, pattern z) have a cleaved, *p*-nitrophenylate exoliposomal head group and an intact benzoate ester endoliposomal head group.

endoliposomal functional head groups to exoliposomal loci, whereas similar treatment of differentiated **1-F**, **2-F**, or **5-F** coliposomes brings about reequilibrations with $t_{1/2}$ = 2–5 min.

Even 1 h of heating at 60 °C occasions only 18% flip of **3-F** or **4-F**. This unprecendented[3,13] thermal stability for ammonium ion lipids, expressed as extraordinary resistance to transverse bilayer migration, reflects the inability of biphenyl-stiffened, bridging **3-F** or **4-F** to readily bend within the bilayer. Monopolar lipids, or the all-methylene bola **1-F** with no built-in barrier to bending, exhibit normal dynamics.

In bilayers, the biphenyl units of **3-F** and **4-F** inhibit bending in the middle of the bolas' main chains. However, *monolayers* of **3-NF**, like the natural bolaamphiphiles,[1a,d,e] do feature U-plan arrangements at the air/water interface.[14] The bending here must occur at either side of the biphenyl group.

Acknowledgment. We are grateful to Mr. J. Simon and Prof. H. Ringsdorf for monolayer experiments with **3-NF**. We thank the U.S. Army Research Office and the Busch Memorial Fund of Rutgers University for financial support.

Supplementary Material Available: Details of synthetic schemes for bolaamphiphiles **3-F** and **4-F** (2 pages). Ordering information is given on any current masthead page.

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Synthesis of a 4-Thio-2′-deoxyuridine-Containing Oligonucleotide. Development of the Thiocarbonyl Group as a Linker Element

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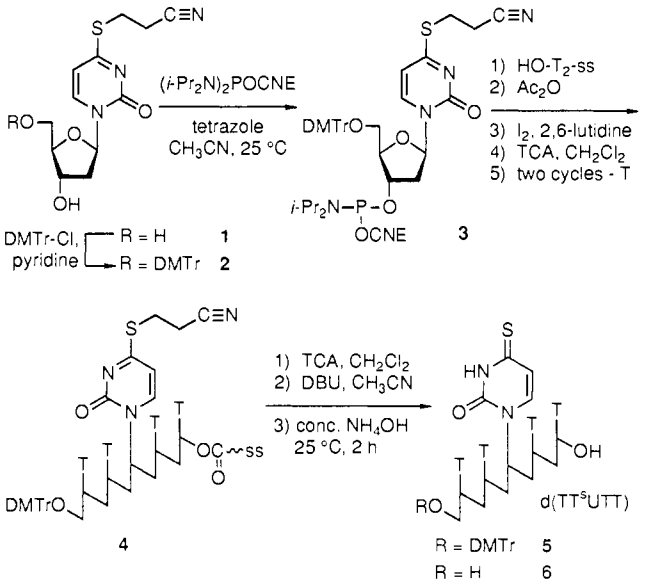
The synthetic incorporation of non-natural functionality into oligonucleotides has provided a variety of templates upon which to tether reactive or reporter groups[2] such as chemically reactive species[3,4] or intercalating ring systems.[5] Various reports have described the synthesis and incorporation of "modified" nucleic acids into oligonucleotides;[2,6] the most flexible approaches have utilized a postsynthesis modification strategy. This tactic involves the incorporation of a functionalized non-natural nucleic acid into a growing oligonucleotide chain and is followed by chemical modification of the non-natural base. This makes possible the divergent incorporation of reactive functionality that would otherwise be incompatible with solid-phase synthesis conditions. Examples include reports by Webb and Matteucci[3a] and Verdine and co-workers[7] that describe the synthesis and postsynthetic modification of base-functionalized oligonucleotides. Herein, we report our preliminary results on the incorporation of 4-thio-2′-deoxyuridine residues into oligodeoxynucleotides,[8] and the development of the appendant thiocarbonyl group as a site-specific handle for the attachment of functionalized tethers.

The synthesis of thionucleic acid-containing oligonucleotides is hampered by the instability of the thiocarbonyl group to solid-phase synthesis conditions.[8a] We reported[9] an efficient synthesis of *S*-(2-cyanoethyl) 4-thio-2′-deoxyuridine (**1**) and detailed its stability to reagents used for oligonucleotide synthesis.[8b,10] An *S*-cyanoethyl ether allows for S-deprotection concomitant with removal of the cyanoethyl ester phosphate protecting groups.[10] Disulfide-based protecting groups were unsuitable, since the disulfide linkage labilized the carbon–sulfur thioimidate bond to hydrolysis. Other protecting groups[8a] and methods for incorporation of a thiocarbonyl group[11] have not proven effective.

Protection of **1** as the dimethoxytrityl (DMTr) ether (DMTrCl, pyridine, 25 °C, 87%) afforded **2** and was followed by phosphitylation[10] (tetrazole, $(i\text{-Pr}_2\text{N})_2\text{POCH}_2\text{CH}_2\text{CN}$, CH_3CN, 25 °C, 98%) to afford phosphoramidite **3**. Incorporation of **3** into a growing oligonucleotide chain was achieved using an Applied Biosystems 380B oligonucleotide synthesizer.[10] Thus, phosphitylation of the 5′-hydroxyl group of a solid support (ss) linked TT-dinucleotide with **3** was followed by standard end-capping (Ac_2O, 2,6-lutidine, THF), oxidation (I_2, H_2O/pyridine/THF), detritylation (2% CCl_3CO_2H (TCA) in CH_2Cl_2), and oligomer elongation with two additional thymidine residues to afford **4**. The *S*-cyanoethyl ether and *O*-cyanoethyl phosphate esters were removed by treatment with 1.0 M DBU in CH_3CN for 1 h.[12] Cleavage of the oligonucleotide from the solid support (concentrated NH_4OH, 25 °C, 2 h) afforded pentamers **5** and **6**. Yields for each coupling step were in excess of 94%. "Trityl-on" pentamer **6** could be purified by HPLC (1 × 25 cm C18 column, 0.1 M NH_4OAc, 1–50% CH_3CN/H_2O gradient, 4 mL/min). The purity of pentamers **5** and **6** was determined by ^1H NMR spectroscopy; no resonances were observed that were attributable to a uridine residue.



(1) Recipient of a Camille and Henry Dreyfus Foundation Distinguished New Faculty Award (1989-94) and an American Cancer Society Junior Faculty Research Award (1991-93).
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