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Click-Chemistry as a Mix-and-Match Kit for Amphiphile Synthesis

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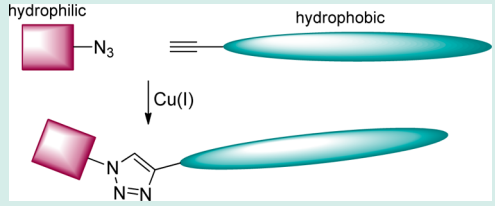
Supporting Information

ABSTRACT: A small library of amphiphilic compounds was synthesized in an array using the Huisgen 1,3-dipolar cycloaddition of terminal alkynes with azides (CuAAC or click reaction). The self-assembling properties of these compounds were evaluated by polarizing microscopy and synchrotron small-angle X-ray scattering analysis.



KEYWORDS: *amphiphiles, cycloaddition, click chemistry, self-assembly, surfactants*

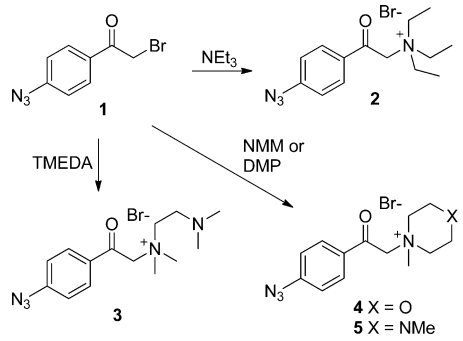
INTRODUCTION

Amphiphilic compounds are industrially significant chemicals that are typically composed of a polar, hydrophilic headgroup and a nonpolar, hydrophobic tail. Commercially important amphiphiles include surfactants, soaps, lipids, and amphiphilic block copolymers, which are used extensively in food, paint, adhesive, detergent, and cosmetic applications.[1] When placed in a solvent, most often of aqueous nature, amphiphilic molecules will not only migrate to surfaces but also self-assemble into a range of structures. These lyotropic liquid crystalline assemblies include structures such as micelles, lamellar bilayers, and hexagonal and cubic mesophases with a range of interfacial curvatures.[2] The nature of the aggregates formed by these amphiphilic moieties varies considerably due to their molecular architecture. The molecule's inherent shape and polarity properties will have a significant effect on the nanostructure and long-range order of the self-assembled aggregates formed.[3−5] Variables, such as the balance of lipophilic and hydrophilic strength of the nonpolar and polar moieties, the critical packing parameter, and the composition of the components, all influence the nature of the self-assembled structure for a given amphiphilic system.[6,7] Local environmental properties will also play a significant role in the phase behavior of these compounds.

One of the more exciting applications for self-assembled macromolecular structures is in the controlled delivery and release of drugs.[8,9] High-throughput techniques for screening the self-assembly properties of amphiphilic molecules can greatly accelerate the discovery of 3D nanostructured nanoparticles for drug delivery.[10] One limitation on the rapid development of this technology is the challenge in synthesis and purification of diverse amphiphiles with which to tune the molecular architecture of the self-assembled nanoparticles. This is especially problematic for research groups without access to specialist synthetic organic chemistry capabilities and equipment. Herein, we describe a modular system of easily prepared, polar head groups and commercially available, nonpolar tail groups that are suitably functionalized to click[11] together in any combination. The final amphiphile assembly uses the operationally simple processes of copper-catalyzed, Huisgen 1,3-dipolar cycloaddition of terminal alkynes with azides (CuAAC or click reaction).[12] This method lends itself to rapid amphiphile library generation for high-throughput characterization.

We chose the hydrophobic tail as the alkyne partner for the CuAAC cycloaddition reaction; primarily because terminal alkynes are commercially available in all appropriate chain lengths for amphiphile synthesis. C10, C12, C14, C16, C18, and C20 straight-chain terminal alkynes were acquired from commercial suppliers. The cationic, hydrophilic headgroup, azide partners for the CuAAC click reaction were prepared by reacting commercially available 4-azidophenacyl bromide **1** with tertiary amines in acetone. Four amines were selected: triethylamine, *N*,*N*,*N*′,*N*′-tetramethylethylenediamine (TMEDA), *N*-methylmorpholine (NMM), and *N*,*N*′-dimethylpiperazine (DMP). The product quaternary ammonium salts **2**−**5** precipitated immediately from acetone and could be collected in essentially pure form by filtration (Scheme 1).

Scheme 1. Synthesis of Hydrophilic Head Group (Azide Partners)



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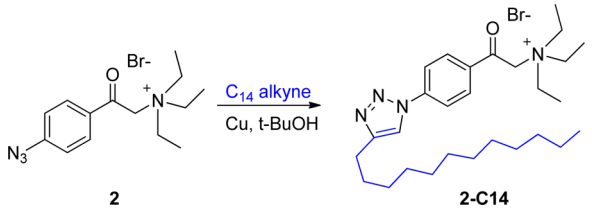
The azide head groups **2**–**5** were then reacted with the alkyne tail groups in the presence of copper metal with *t*-butanol as solvent (for example, Scheme 2). The reactions were

Scheme 2. Click Reaction between Azide Head Group 2 and C14 Alkyne Tail (1-Tetradecyne)



2 **2-C14**

carried out simultaneously in a six by four array of sample tubes set in a machined aluminum block (Figure 1), heated at 35 °C

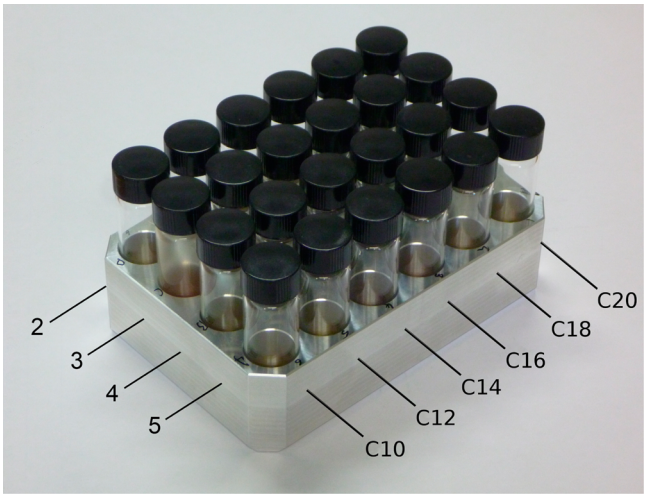


Figure 1. Amphiphile library synthesis array. Azides **2**–**5** reacted with terminal alkynes (C10 = 1-decyne, C12 = 1-dodecyne, C14 = 1-tetradecyne, C16 = 1-hexadecyne, C18 = 1-octadecyne, C20 = 1-icosyne).

for 24 h. The reactions proceeded to full conversion, as monitored by LC–MS in purities ranging from 79 to 100% (average 89%). The copper catalyzed azide–alkyne cycloaddition reaction is known to proceed regioselectively to give 1,4-cycloadducts.[12] Excess copper was simply filtered off, then the solvent was evaporated and the solid amphiphile products were analyzed as described below. The triazole cycloadducts are henceforth referred to by a combination of their headgroup number (**2**–**5**) and precursor alkyne chain length (**C10**, **C12**, **C14**, **C16**, **C18**, and **C20**).

Ultimately, as these amphiphiles are aimed at the creation of self-assembled phases, the utility of the material library can be assessed by considering each material's aggregation properties in aqueous solutions. Assessing self-assembly and the structures formed can be performed via a range of techniques. Cross-polarized light microscopy (CPLM) can differentiate between isotropic and anisotropic liquid crystalline phases; characteristic optical textures allow visual identification of certain phases.[13] Definitive phase assignment is based on small-angle X-ray scattering (SAXS) data which yields structural information as well as unit cell size.[2]

The self-assembly in water of each amphiphile was initially assessed using polarizing microscopy through water-penetration temperature scans. Self-assembly was observed for all lipids in the presence of water, with lamellar and micellar phases typically observed. This is expected as the charged head groups and straight saturated aliphatic chains will often lead to flat interfaces. In general, the longer chain lipids formed lamellar structures, while the shorter C10 and C12 lipids typically formed micellar aggregates at room temperature under excess water conditions.

Several trends in self-assembly behavior were observed as a function of increasing aliphatic chain length. Typically, the shorter chained amphiphiles rapidly formed micelles at the water interface at room temperature. At room temperature, a clear phase sequence of water, micelles, lamellar phase, inverse micelles, and a second lamellar phase (possibly a re-entrant lamellar phase) were observed for samples **2-C10**, **2-C12**, **2-C14**, **3-C10**, and **5-C10**. Representative microscopy data for these samples are provided in Figure 2 (all of the microscopy

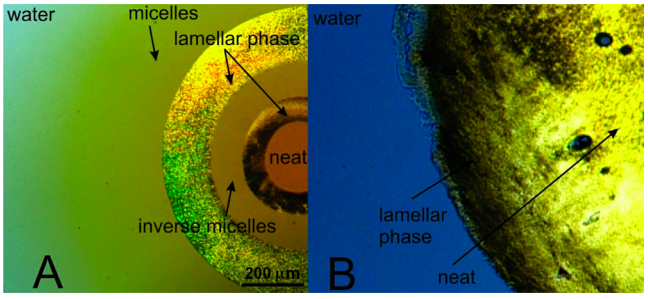


Figure 2. CPLM images of water penetration scans at 35 °C for (A) **2-C10** and (B) **2-C20**. Birefringent lamellar and isotropic domains are highlighted.

data is available in the Supporting Information). This sequence is as expected with decreasing water content. As the hydrophobicity of the molecules increased, lamellar phases were observed to exist in excess water and these retained their integrity to relatively high temperatures (50–60 °C). As the temperature was increased, a transition from a lamellar to a micellar phase was observed. The systematic increase in chain length led to a distinct increase in aggregate stability; the lamellar–fluid isotropic phase transition temperature increased with increasing chain length. Structural differences in the headgroup of the amphiphile also affect the stability of the lamellar aggregate with temperature. Series **3**, **4**, and **5** retained their lamellar structure at lower chain lengths and higher temperature than series **2**. This may be correlated to the level of hydrogen bonding and "headgroup to headgroup" or "headgroup to solvent" interactions as well as the relative hydrophilicities of the head groups. Only the triethylamine headgroup has one hydrogen bond donor/acceptor while the others have at least two. Using the octanol–water partition coefficient, it is possible to assess the hydrophilic nature of each headgroup and relate this to the persistence of the lamellar phase at higher temperatures. The most hydrophilic headgroup is that of triethylamine (series 2) with an octanol–water partition coefficient log $k_{o/w}$ 1.44. The other head groups, tetramethylethylenediamine, *N*-methylmorpholine, and 1,4-dimethylpiperazine, have octanol–water partition coefficients of 0.30, −0.33, and −0.40, respectively. Therefore a lower log $k_{o/w}$ is associated with increased stability of the liquid crystalline (specifically lamellar) phases and their retention to higher

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temperatures. In addition, lamellar phases were observed at shorter chain lengths for molecules with a lower log $k_{o/w}$.

Synchrotron small-angle X-ray scattering (SAXS) was also performed on these materials under excess water conditions to confirm the microscopy findings and evaluate the effect of molecular structure on the adopted mesophase and associated lattice parameter. SAXS data confirmed the presence of lamellar phases under excess water conditions (80 wt % water) observed via CPLM for the longer chained lipids. A broad, diffuse peak, characteristic of a fluid isotropic phase (potentially micellar or liposomal material), was commonly present in these highly hydrated samples at 25 °C.

A clear increase in long-range ordering was observed with increasing chain length for all head groups tested under excess water conditions (80 wt % water). Figure 3 shows a typical progression from broad diffuse peaks indicative of micelle formation observed for short-chain amphiphiles to distinct Bragg reflections indicative of lamellar phase formation by longer chain amphiphiles. This trend was observed for all four headgroup series in agreement with the CPLM data presented above (see the Supporting Information for SAXS of all molecules in 80% water conditions).

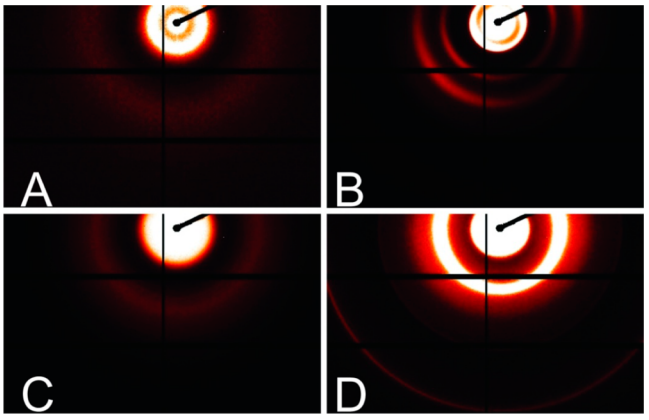

Figure 3. Diffraction patterns of (A) **2-C10**, (B) **2-C20**, (C) **4-C10**, and (D) **4-C20** at 30 °C. Images A and B show broad micellar peaks at low angle, with limited long-range ordering, while image C shows a lamellar phase with a lattice parameter of 122 Å and image D a lattice parameter of 133 Å.

In addition, for a particular headgroup, increasing chain length also drives an increase in lattice parameter of the lamellar phase (for constant hydration level and temperature). An increase in lattice parameter was observed with increasing chain length for the C16, C18, and C20 derivatives (see Figure 4).

Construction of self-assembling material libraries has been previously attempted; however, generating sufficient numbers of related materials with controlled structural differences remains limited. For example, in the comprehensive review by Fong et al., relatively few materials that form inverse phases were identified, and these often had limited structural information available.[14] At the same time, the CuAAC click reaction has been extensively used for generating libraries of compounds, especially in the pharmaceutical[15] and materials[16] fields. It is therefore somewhat surprising that this versatile and operationally simple method has not to date been exploited more widely in assembling surfactant libraries. We are currently exploring CuAAC click methodology to prepare amphiphile libraries with diverse polar groups such as sugars, peptides, and anionic and cationic head groups, along with a greater variety of hydrophobic tail groups.

EXPERIMENTAL PROCEDURES

General synthetic procedures, and analytical instrumentation were as previously described.[17]

Representative Procedure for Headgroup Synthesis. 2-(4-Azidophenyl)-*N*-(2-(dimethylamino)ethyl)-*N*,*N*-dimethyl-2-oxoethan-aminium bromide **3**. A flame-dried 2−5 mL microwave vial was charged with 1-(4-azidophenyl)-2-bromoethanone (100 mg, 0.42 mmol) and acetone (1 mL). The vial was capped and the reaction cooled in an ice bath. *N*,*N*,*N*,*N*-Tetramethylethane-1,2-diamine (0.42 mmol, 61 μL) was then added, dropwise, whereupon a white precipitate formed almost immediately. Acetone (0.5 mL) was added, and the reaction stirred at room temperature for 2 h. The white precipitate was

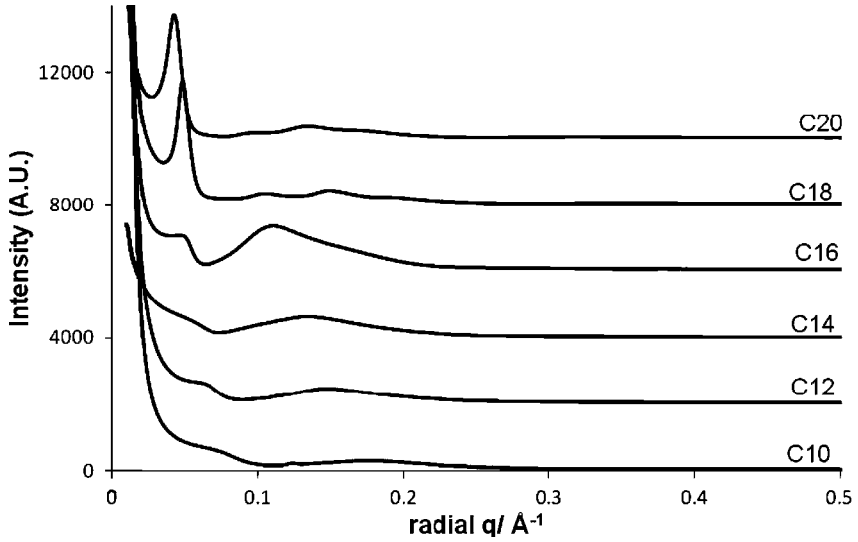

Figure 4. Diffraction patterns of compounds **5-C10** to **5-C20** at 30 °C. **5-C10**, **5-C12**, and **5-C14** show broad micellar diffraction peaks with very limited long-range order while the C16−C20 derivatives adopt lamellar phases at 80 wt % water with lattice parameters of 120, 124, and 144 Å for C16, C18, and C20, respectively. Traces have been artificially offset in the *y*-axis for clarity.

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then gently broken up with a glass pipet, collected, and washed with acetone to give the bromide salt **3** (136 mg, 91%) as an off-white solid. ^{1}H NMR (D_2O, 400 MHz) δ 7.85 (1H, d, J = 8.7 Hz); 7.13 (1H, d, J = 8.7 Hz); 3.67 (2H, t, J = 6.6 Hz); 3.29 (3H, s); 2.68 (2H, t, J = 6.6 Hz); 1.95 (3H, s). ^{13}C NMR (D_2O, 200 MHz) δ 188.8, 146.5, 130.5, 129.6, 119.3, 60.4, 53.3, 51.3, 43.5. HRMS (ESI) Found 276.1812; $C_{14}H_{22}N_5O^+$ requires 276.1824.

Procedure for Amphiphile Library Synthesis. Each of 24 glass vials (18 mm × 50 mm) in a 4 × 6 array in an aluminum reaction block was equipped with magnetic stirrer bars. Each vial was charged with an amphiphilic headgroup (15 mg), tail group (1 equiv), *t*-BuOH (0.9 mL), and H_2O (0.45 mL). The reaction block was heated to 35 °C, and the reactions were stirred for 10 min to ensure complete dissolution of the starting material. Copper powder (~200 mg) was added to each vial. The vials were then capped and the reactions stirred at 35 °C for 24 h. After this time, EtOH (2 mL) was added to each vial and the reactors stirred for an additional 10 min. The reactions were then transferred to a Whatman Unifilter (10 mL, 24 well polypropylene GF/C filter) and the mother liquor collected in 18 mm × 50 mm glass vials. The solvent was removed in a Genevac EZ-2 Plus to give the desired amphiphiles. The residues were subsequently placed in a vacuum oven at 50 °C for 2 h, then analyzed for purity by LC–MS (see the Supporting Information).

Synchrotron Small Angle X-ray Scattering. The internal liquid crystalline structure of the hydrated phases was determined using small-angle X-ray scattering (SAXS). Data were collected using the SAXS/WAXS beamline at the Australian Synchrotron using a beam with wavelength λ = 1.033 Å (12.0 keV) with a typical flux of 10^{13} photons/s. 2D diffraction patterns were recorded on a Decris-Pilatus 1 M detector of 10 modules. The detector was offset to access a greater q-range. A silver behenate standard (λ = 58.38 Å) was used to calibrate the reciprocal space vector. The samples were loaded in special glass 1.5 mm capillaries (Hampton Research) and positioned in a custom designed semihigh throughput capillary holder capable of holding 40 capillaries with temperature controlled to ±0.1 °C between 20 and 75 °C. Temperature control was via a recirculating water bath. Exposure time for each sample was 1 s. Data were analyzed using aXcess software created by Andrew Heron at Imperial College, London.[4] Samples, typically 3–4 mg of amphiphile were hydrated with 80 wt % water 24 h prior to data collection. Because of the small sample size, data could not be obtained at higher temperatures due to sample drying.

Cross-Polarization Microscopy. A small amount of neat amphiphile was placed onto a microscope slide and melted so as to form a uniform air/sample interface. A coverslip was placed over the melted amphiphile which was cooled to room temperature prior to hydration of the material. The microscope slide was placed into a Linkam PE94 hot stage (Linkam Scientific Instruments Ltd.; Surrey, England) and heated at 1 °C/min or less. The interaction of water and the amphiphile was observed with a Nikon Eclipse 80i inverted microscope (Coherent Scientific, Melbourne) without and with an analyzer. Images were captured with a Nikon DS-Fi1 camera (Coherent Scientific, Melbourne).

The logarithmic values of water/octanol partition coefficients (log $K_{o/w}$) were obtained from the online database of the Sangster Research Laboratories (http://logkow.cisti.nrc.ca/logkow/).

■ ASSOCIATED CONTENT

Supporting Information

Experimental details, NMR spectra of compounds **2**–**5**, HPLC purity analysis, cross-polarized light microscopy data, and synchrotron small-angle X-ray scattering data. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

GPS conceived the project, OEH and XM designed and conducted experiments, GPS and XM co-wrote the manuscript.

Notes

The authors declare no competing financial interest.

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