ABSTRACT SUMMARY
A high-throughput (HT) method for the synthesis and phase characterization of a library of sugar-based amphiphilic compounds was developed. A combinatorial approach was employed to synthesize an amphiphile library with systematic variations in chain length, unsaturation and branching. HT small-angle X-ray scattering (SAXS) was utilized to ascertain the liquid-crystalline phase behaviour of these compounds. The results have been used to search for novel amphiphiles with the desired liquid-crystalline characteristics for drug delivery, as well as to increase our understanding of the structure-property relationship that govern self-assembly.

INTRODUCTION
Despite the discovery of numerous glycans (sugars that bind to proteins), only one small-molecule sugar-based drug is found in the top 100 pharmaceutical bestsellers. This is perhaps unsurprising, when considering the challenges in sugar-based drug design; sugars are highly polar, and therefore difficult to absorb, and they are quickly removed from the circulation. A potential approach to overcome these problems, is to formulate sugar-based drugs as self-assembling amphiphiles. However, in order to form such therapeutics, we first need to understand how and why self-assembly occurs.

We used the copper catalysed azide-alkyne cycloaddition (CuAAC) ‘click’ reaction to synthesize a small library of single-chained sugar amphiphiles, and have since expanded this library to cover double and triple-chain amphiphiles, with systematic variations in headgroups, chain lengths and chain unsaturation. A HT SAXS screen at the Australian Synchrotron was subsequently carried out, to search for trends in liquid-crystalline phase behaviour. For drug delivery applications, inverse cubic and hexagonal phases are desired, since these can be dispersed into nanoparticles. Therefore, this data can be used to telescope the search for drug-like sugar amphiphiles that have appropriate phase behaviour for nanoparticle delivery.

EXPERIMENTAL METHODS
General procedure for the HT synthesis of amphiphiles. To each of 24 glass vials (18 mm × 45 mm) in a 4 × 6 array aluminium reaction block, was added a solution of azido-sugar (1.0 eq., ~ 15 mg) in 2:1 t-BuOH:water (1.5 mL). Alkyne tail (1.0 eq.) was added and the reaction block heated, with stirring, to 40 °C. After dissolution, copper powder (~200 mg) was added and the reaction stirred for 24-48 h. The reaction mixture was cooled, diluted with ethanol (2 mL) and filtered through Celite® into 24 glass vials (25 mm × 75 mm). Concentration in vacuo on a Genevac EZ-2, followed by vacuum oven drying (50 °C, 3h), afforded the amphiphile products. The amphiphiles were characterised using MALDI-TOF, HT 13C NMR and ICP-MS.

Synchrotron Small Angle X-Ray Scattering (SSAXS). Approximately 0.6 mg of each amphiphile was dispensed into a 96-well plate and an excess of water (60% w/v) was added to each well using a Mosquito® liquid dispensing robot. A duplicate of each plate was left dry to ascertain the phase behaviour of the neat sample. The samples were equilibrated under controlled conditions of temperature and humidity for 5 days before SAXS analysis was carried out. Samples were analysed within the 96-well plate using a bespoke sample holder at the SAXS beamline at the Australian Synchrotron. Each plate was analysed at temperatures between 25 °C and 55 °C. The number and distribution of reflections was used to assign the mesophase at that given temperature.

RESULTS AND DISCUSSION
Single, double and triple chained clickable hydrophobic tails were synthesized in a combinatorial manner (Scheme 1), while azido-sugar headgroups were commercially available or prepared in a short synthesis from the parent sugar.
Scheme 1: Tail synthesis; R groups include (CH$_2$)$_n$CH$_3$, (CH$_2$)$_n$CH=CH(CH$_2$)$_m$CH$_3$ and phytanly (branched).

These two component types were ‘clicked’ together in a HT array to yield 57 single chain, 46 double chain and 35 triple chain amphiphiles, in a total library of 138 amphiphiles (Scheme 2).

Liquid-crystalline phase characterization was conducted using a HT SAXS screen at the Australian Synchrotron. Diffraction patterns of the amphiphiles were collected neat and at 60% hydration, to determine their thermotropic and lyotropic phase behavior.

Single chain amphiphiles were found to form ‘normal’ liquid-crystalline phases (ie the amphiphiles pack and curve away from water) or lamellar phases. While triple chain amphiphiles tended to form inverse micelles due to the very large chain spaly.

Double chain tails proved to be highly successful for the formation of inverse cubic and hexagonal phases (which are suitable for drug delivery). Saturated, short chain monosaccharides mostly formed inverse gyroid phases (Figure 1), while longer chain saturated, and mono-unsaturated chains, tended to form inverse hexagonal phases.

Figure 1: Diffraction pattern of the xylose amphiphile shown, at 37 °C and 60% (w/v) water showing the formation of an inverse bicontinuous gyroid cubic phase. Assigned Bragg reflections are identified.

The validity of our screen was verified by resynthesis of an amphiphile identified as having interesting phase behavior on a preparative scale, followed by full phase characterization.

CONCLUSION
A library of single, double and triple chain sugar-based amphiphiles were synthesised in a HT manner. Our HT SAXS results showed that desired drug delivery phases can be achieved with a variety of sugars, by manipulating the number of side chains, as well as the size and degree of unsaturation of the chain.

REFERENCES

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