A modular approach towards drug delivery vehicles using oxanorborne-based non-ionic amphiphiles†

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The self-assembly of non-ionic amphiphiles with a hydroxylated oxanorborne head-group was controlled using amino acid units as spacers between hydrophilic and lipophilic domains to get spherical supramolecular aggregates. The ability of these systems to harbour therapeutic agents like ibuprofen, and their drug-release profiles were evaluated. Apart from directing the assembly, the intervening amino acid unit was found to help in drug entrapment as well. The presence of cholesterol improved their drug-loading ability, and an encapsulation efficiency of up to 66% was shown by the formulation containing the phenylalanine residue as the spacer (NC1c). There was no burst release, and 45% drug release was observed at the end of 24 h in this case (cf. soyaphosphatidylcholine based formulation = 49%). The results from SEM, Cryo-TEM, PXRD and confocal microscopic studies with some insights into molecular packing in this class of aggregates are also included.

Introduction

Drug delivery systems have become indispensable in therapy and can address problems like low stability, taste, solubility, site-specificity, drug toxicity and bioavailability. They belong to different structural classes like lipids, polymers or interpenetrating supramolecular networks involving one or more of these molecular systems.1–4 Depending upon the requirement, they can be developed for oral as well as parenteral applications. Apart from simple liposomes, a number of their variants have emerged during the past several years which include phytosomes, transfersomes, ethosomes, archaeosomes, niosomes etc.5–11 They differ mainly in the nature of lipid components and other constituents/additives introduced to modulate elasticity, permeability, drug-loading and -release profiles. Niosomes, which are based on non-ionic amphiphiles, offer many advantages over liposomes like: (i) better stability, (ii) long shelf-life, (iii) lower cost of production, (iv) lower toxicity, (v) better BBB permeation and (vi) stability towards opsonisation and a lower rate of drug clearance.12–16 Archaeosomes are related systems which use archaeal membrane components for drug encapsulation.17–21 The ability of extremophiles in an archaean domain to live under conditions like high salinity, acidity, temperatures or pressures is attributable to a special type of glycolipids in their outer membranes.22 Generally, isoprenoid alkyl chains, either alone or in combination with cyclopropane rings linked to sn-2,3 carbons of glycerol form the lipophilic part, and β-galactofuranosyl units are seen as the polar head. Although they can find tremendous application in drug delivery, the main bottle-neck lies in the difficulty in isolation from natural sources in pure form, and large scale production. Gaining inspiration from archaeal lipids, we have started a program to develop their structural mimics using a hydroxylated oxanorborne skeleton as the polar head. The 2⁻ hydroxyl groups at C-5 and C-6 (Fig. 1A), primary OH at C-1, and the bridging oxygen O-7 give the appearance of a furanose unit, while the hydroxyl groups at C-2 and C-3 in parallel arrangement are available for alkyl chain attachment. Our previous studies have shown the remarkable ability of such systems to self-assemble to give different hierarchical structures.23,24 A notable feature in their assembly was the continuous array of hydrogen bonding interactions involving donor–acceptor (D–A) groups on the head group as shown in Fig. 1B. Hierarchical preference during the self-assembly of achiral amphiphiles in this series was attributed to a twist in the orientation of the head group during assembly.25 This indicated that a chiral environment in the immediate vicinity of the head-group would help in controlling the aggregation profile. Based on this, we have designed a new series of amphiphiles, incorporating amino acid residues as spacers to link the hydrophilic and lipophilic...
domains (Fig. 1C). Apart from chirality, they provide different side chain groups which bring specific steric and electronic influences during self-assembly and drug encapsulation (vide infra). Details of these studies and the results are presented below.

Experimental section

Materials

Acid chlorides (octanoyl and myristoyl chlorides) and maleic anhydride were purchased from Aldrich. Furans, N,N-diisopropylethylamine and EDC–HCl were purchased from Spectrochem, and the amino acids used in the present study are from Avra, SRL and Spectrochem brands. The drug ibuprofen was a gift sample from Shasun Pharmaceuticals Ltd. Lechitin Soya (phosphatidylcholine) from HiMedia, cholesterol from SDFCL, and fluorescein from Aldrich were also used in our studies. The dialysis bag (MWCO 12 000–14 000) was purchased from Spectrochem, ethylamine and EDC/C01428anhydride were purchased from Aldrich. Furan, N-acid chlorides (octanoyl and myristoyl chlorides) and maleic anhydride, treatment of the resulting adduct with ethanolamine to get the imide intermediate, its esterification with appropriate N-acylated amino acids and a final cis-dihydroxylation step. Specific details of these synthetic steps are given in the ESI† (Scheme SI-1). Spectral data of products 1a–e which had aggregation characteristics suitable for drug delivery studies are given below. Data of others are included in the ESI.†

Compound 1a. Yield, 92%; Rf (EtOAc), 0.40; 1H NMR (CDCl3, 500 MHz): δ 4.61 (s, 2H), 4.43 (bs, 1H), 4.30 (t, 2H, J = 5.5 Hz), 3.94 (s, 2H), 3.92 (s, 2H), 3.75 (t, 2H, J = 5.0 Hz), 3.40 (bs, 1H), 2.83 (2H), 2.21 (t, 2H, J = 7.5 Hz), 1.60 (quin, 2H, J = 7.5 Hz), 1.29–1.23 (m, 8H), 0.86 (t, 3H, J = 7 Hz), –NH proton did not appear; 13C NMR (CDCl3 + CD3OD, 125 MHz): δ 176.2, 173.5, 172.7, 84.3, 73.3, 65.3 cm−1; IR (KBr): 3322, 2921, 2850, 1709, 1548, 1438, 1405, 1326, 1190, 1119, 998, 879, 849, 828, 813, 733, 653 cm−1; HRMS (ESI) exact mass calcd for C20H32N2O8 (M + H)+ 441.2237, found (M + H)+ 441.2233.

Compound 1b. Yield, 96%; Rf (EtOAc), 0.40; 1H NMR (CDCl3, 500 MHz): δ 6.15 (d, 1H, J = 7.6 Hz), 4.65 (s, 1H), 4.62 (s, 1H), 4.52 (quin, 1H, J = 7.2 Hz), 4.38–4.33 (m, 1H), 4.26–4.20 (m, 1H), 4.04 (bs, 1H), 4.00–3.92 (m, 3H), 3.83–3.70 (m, 2H), 2.86 (d, 2H, J = 2.4 Hz), 2.22–2.18 (m, 2H), 1.60 (quin, 2H, J = 6.8 Hz), 1.34 (d, 3H, J = 7.2 Hz), 1.30–1.22 (m, 8H), 0.87 (t, 3H, J = 6 Hz); 13C NMR (CDCl3, 100 MHz): δ 176.2 (2C), 173.5, 172.7, 84.3, 84.2, 73.1 (2C), 61.3, 48.0, 45.6, 45.5, 38.2, 36.7, 31.8, 29.3, 29.1, 25.7, 22.7, 18.4, 14.2; IR (KBr): 3442, 3056, 2985, 1709, 1548, 1428, 1267, 897, 755 cm−1; HRMS (ESI) exact mass calcd for C21H33N2O8 (M + H)+ 441.2237, found (M + H)+ 441.2236.

Compound 1c. Yield, 91%; Rf (EtOAc), 0.50; 1H NMR (CDCl3, 500 MHz): δ 7.30–7.20 (m, 3H), 7.09 (d, 2H, J = 7.5 Hz), 6.00 (bs, 1H), 4.81 (quin, 1H, J = 7.5 Hz), 4.66 (d, 1H, J = 9.0 Hz), 4.61 (s, 1H), 4.42–4.30 (m, 1H), 4.20–4.14 (m, 1H), 4.00–3.92 (m, 2H), 3.81–3.68 (m, 2H), 3.10 (dd, 1H, J = 14 Hz, 5.5 Hz), 2.99 (ddd, 1H, J = 14 Hz, 6.5 Hz, 2 Hz), 2.82 (s, 2H), 2.15 (sext, 2H, J = 7 Hz), 1.57–1.49 (m, 2H), 1.32–1.20 (m, 8H), 0.87 (t, 3H, J = 5.5 Hz), –OH protons did not appear; 13C NMR (CDCl3, 125 MHz): δ 176.2, 173.6, 171.2 (2C), 136.0, 129.4 (2C), 128.7 (2C), 127.2, etc.
84.24, 84.20, 73.1, 61.3, 52.9, 45.6, 45.5, 38.0, 37.8, 36.6, 31.8, 29.8, 29.2, 29.1, 25.7, 22.7, 14.2; IR (KBr): 3426, 3057, 2930, 2861, 1780, 1746, 1708, 1653, 1429, 1337, 1266, 1190, 1115, 1010, 898, 820, 744 cm$^{-1}$; HRMS (ESI) exact mass calculated for C$_{27}$H$_{37}$N$_2$O$_8$ (M + H)$^+$ 517.2550, found (M + H)$^+$ 517.2561.

**Compound 1d.** Yield, 89%; $R_f$ (EtOAc), 0.44; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 6.04 (d, 1H, $J = 8.8$ Hz), 4.66 (s, 1H), 4.60 (s, 1H), 4.48–4.41 (m, 2H), 4.14–4.10 (m, 1H), 3.98 (d, 1H, $J = 6.0$ Hz), 3.94 (d, 1H, $J = 5.6$ Hz), 3.86–3.79 (m, 1H), 3.75–3.70 (m, 1H), 2.85 (s, 2H), 2.24 (sext, 2H, $J = 4$ Hz), 2.10 (sext, 1H, $J = 6.4$ Hz), 1.65–1.55 (m, 2H), 1.35–1.20 (m, 8H), 0.91 (d, 3H, $J = 6.8$ Hz), 0.88–0.84 (m, 6H), $-$2OH protons did not appear; $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 176.1 (2C), 174.1, 171.7, 84.3, 84.2, 73.1, 73.0, 60.9, 56.9, 45.6, 45.5, 38.0, 36.9, 31.8, 31.2, 29.8, 29.3, 29.1, 25.8, 22.7, 19.2, 17.7, 14.2; IR (KBr): 3661, 3269, 3056, 2928, 2860, 2360, 2312, 1709, 1518, 1429, 1266, 1193, 1009, 897, 746 cm$^{-1}$; HRMS (ESI) exact mass calculated for C$_{27}$H$_{37}$N$_2$O$_8$ (M + H)$^+$ 469.2550, found (M + H)$^+$ 469.2570.

**Compound 1e.** Yield, 95%; $R_f$ (EtOAc), 0.5; $^1$H NMR (CDCl$_3$, 400 MHz): $\delta$ 5.95 (d, 1H, $J = 9.2$ Hz), 4.66 (s, 1H), 4.60 (s, 1H), 4.55 (dt, 1H, $J = 9.2$, 4.8 Hz), 4.41–4.35 (m, 1H), 4.19–4.14 (m, 1H), 3.97 (q, 2H, $J = 6$ Hz), 3.84–3.61 (m, 2H), 2.85 (d, 2H, $J = 1.6$ Hz), 2.21 (dt, 2H, $J = 7.2$, 4.0 Hz), 1.65–1.55 (m, 4H), 1.44 (sext, 2H, $J = 9.2$ Hz), 1.35–1.20 (m, 8H), 0.93 (d, 6H, $J = 6.8$ Hz), 0.87 (t, 3H, $J = 6.5$ Hz) $-$OH proton did not appears; $^{13}$C NMR (CDCl$_3$, 100 MHz): $\delta$ 176.2 (2C), 173.8, 172.7, 84.3, 84.2, 73.1 (2C), 61.1, 50.6, 45.6, 45.5, 41.6, 38.1, 36.7, 31.8, 29.3, 29.1, 25.7, 25.0, 23.0, 22.7, 21.8, 14.2; IR (KBr): 3659, 3430, 3057, 2930, 2863, 1708, 1519, 1430, 1400, 1335, 1267, 1194, 1111, 1010, 898, 818, 742, 611 cm$^{-1}$; HRMS (ESI) exact mass calculated for C$_{27}$H$_{37}$N$_2$O$_8$ (M + H)$^+$ 483.2628, found (M + H)$^+$ 483.2644.

**Niosome preparation.** Niosomes were prepared using thin-film hydration method. Towards this, the lipid (1a–e) alone or in combination with cholesterol and ibuprofen in 1 : 0.5 : 1 ratio was dissolved in methanol. The solvent was then removed by rotary evaporation under reduced pressure to get a thin film which was subsequently hydrated using phosphate buffer (pH 7.2). The resulting suspension was sonicated and then extruded through 1000 nm filters to get more-or-less uniformly-sized niosomes. Cholesterol is known to increase the rigidity of the lipid film which was subsequently hydrated using phosphate buffer (pH 7.2). The resulting suspension was sonicated and then extruded through 1000 nm filters to get more-or-less uniformly-sized niosomes. Cholesterol is known to increase the rigidity of the lipid film and lower the drug release rate. It is generally used as a stabilizer in different lipid-based formulations. 26,27 Soyaphosphatidylcholine-based formulation in the same lipid–stabilizer ratio was used as a reference in our experiments.

**Analysis of size and morphology of nano-aggregates.** Sizes of niosomes were measured mainly using the DLS method (Malvern Zetasizer nano series at 25 °C, in a cell of 1 cm path length). The wavelength of the laser used was 632.8 nm and the scattering angle was 90°. One of the ibuprofen formulations (NC1c) which showed a better performance was analyzed also using qNano (iZON) which works on the Coulter principle. The equipment has an upper and lower fluid cell fitted with silver–silver chloride electrodes. Initially, a membrane of the required pore-size (NP1000, NP800 and NP300 from iZON) was fitted, stretched appropriately and the lower fluid cell was filled with the electrolyte. On applying voltage across the electrodes, ions move between the cells and give a base line current. After calibration with particles of a standard size, the sample (40 µL, prepared in PBS) was loaded onto the upper fluid cell. As the aggregates from the sample move through the nanoparticles, there would be a temporary decrease in current which can be measured. It takes particle by particle measurements, and gives information on the particle size distribution in the samples.

**Drug entrapment and drug release studies.** The suspension containing the drug-loaded samples NC1a–c, Nia–e and LCPC (1 mL) was first centrifuged at 10 000 rpm at 4 °C for 1 h and the unentrapped drug in the supernatant was quantified by noting its absorbance at 223 nm using a UV spectrophotometer. The absorbance was converted to concentration per mL using a standard calibration curve. The percentage of drug encapsulated was calculated using the following equation:

Encapsulation efficiency = (drug encapsulated/total drug) × 100

To evaluate the drug release, pellets of NC1a–e and LCPC (liposome of soyaphosphatidylcholine) formed via centrifugation at 10 000 rpm at 4 °C for 2 h were placed in previously activated dialysis tubing (HiMedia with MWCO 12 000–14 000), and immersed in a phosphate buffer of pH 7.2. The temperature during the experiment was maintained at 37 ± 0.5 °C to simulate physiological conditions. 1 mL each of the samples were then withdrawn at 0.5, 1, 2, 3, 4, 5, 6, 10, 12 and 24 hours, and replaced with an equivalent amount of buffer. These samples were analyzed spectrophotometrically at 223 nm, and from the absorbance values, the percentage of drug released at each of these time points was calculated using a calibration curve.

**Preparation of the sample for confocal microscopy.** The sample was prepared using a thin film hydration method 26,29 using the lipid 1c, cholesterol and fluorescein in the ratio 1 : 0.5 : 1. The film was hydrated using Milli-Q water and the resulting suspension was sonicated and extruded through a 1000 nm membrane filter. 1 mL of this sample was centrifuged at 12 000 rpm at 4 °C for 1 h to remove unentrapped dye. It was suspended in Milli-Q water and a drop was placed on a microscope slide and observed under a confocal microscope.

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To understand dye distribution, fluorescence intensity measurements and multi-section observations were performed.

Results and discussion

Compounds used in the present study (Fig. 2) were synthesized through a multi-step protocol which started with the cycloaddition of furan with maleic anhydride. Treatment of the resulting adduct with ethanolamine gave the corresponding imide (Scheme SI-1, ESI†). The primary hydroxyl of the product was subsequently esterified with appropriate N-acylated amino acids and the conjugates were subjected to cis-dihydroxylation using OsO4 to get the products 1a–e and 2a–e in 35–45% overall yields. All these compounds were characterized using 1H-, 13C NMR, IR spectroscopy, and mass spectrometric methods. They differ in the nature of the amino acid side chain (Me, Bn, iPr, iBu) as well as the hydrocarbon chain (C7/C13) which allowed us to make a comparative assessment of their structure–aggregation relationships.

SEM analysis of samples of 1a–e prepared by drop-casting their acetone solution (1 mg in 1.5 mL) on a silica substrate showed that 1b–e with a C7 alkyl chain have a high tendency to form spherical aggregates with diameters in the range of 300 nm to 5 μm (Fig. 3B–E). In comparison, the glycine derivative 1a (Fig. 3A) was fibrous, suggesting that the side chain groups have an influence on aggregation in this particular solvent. Samples of 1b–e in acetone, when analyzed using DLS, showed the diameters of particles to be in the range of 2–4 μm (Fig. SI-1, ESI†). Upon increasing sample concentration, preference towards spherical aggregates remained intact but their sizes increased gradually reaching up to 6 μm in the case of 1c (Fig. SI-2, ESI†). Except 1b, all others in the C-7 series gave spherical aggregates from their methanol solution as well (Fig. SI-3, ESI†) with a particle size distribution in the range of 500 nm–5 μm (based on SEM studies). Collectively, these observations indicate that the amino acid spacer helps in bringing a conformational bias in the molecule during aggregation and they have a larger preference to form spherical aggregates in solvents like acetone and methanol.

In fact, a simple Boc-group at the terminus (3a–f, Fig. 2) was also sufficient to form spherical aggregates which become evident from SEM images of these derivatives presented in the ESI† (Fig. SI-4); the phenylalanine derivative 3e was the only exception in this group, which gave fibrous aggregates. Their DLS histograms with details of size distributions (400 nm–5 μm) are also given in the ESI† (Fig. SI-4). Interestingly, longer chains in the N-acyl series (2a–e, Fig. 2) did not favour spherical aggregation, and their samples, drop-cast from acetone and methanol, gave fibrillar, flake-like or irregular structures as per SEM (Fig. 3F, Fig. SI-5 and SI-6, ESI†).

Based on this preliminary information from the morphological studies, lipids 1a–e were subjected to detailed investigations. Normally, lipids assemble into normal or reverse micellar structures depending upon the medium. These primary structures can further develop into a lamellar form, or close into vesicular structures. These are dynamic and the preference for one over the other depends on factors like the structure of the lipid, chemical environment, temperature etc.10,31 In the case of non-ionic amphiphiles, lamellar, cubic and hexagonal mesophases have been reported.32–34 As mentioned, their spherical aggregates called niosomes are well known for drug delivery applications.

To gain insight into the nature of aggregates from acetone, the samples of Phe and Val derivatives (1c and 1d) were subjected to TEM analysis. An image of the sample from 1c shown in Fig. 4 appears like that of a vesicle with a boundary of ~20 nm thickness. However, a multi-lamellar vesicle with an aqueous interior is not a possibility as it is formed from acetone. Considering the nature of these lipids and the sample preparation conditions, solid lipid particles (SLPs) are more likely and the distinct periphery could be due to changes during sample drying/staining. This however needs to be verified by analysis of a greater number of samples. Similar images from the sample of 1d are given in the ESI† (Fig. SI-7).

Hydrophilic–Lipophilic Balance (HLB) values for compounds 1a–e were calculated using Griffin’s method35 and were found to be 9.29, 9.00, 7.67, 8.46 and 8.22 respectively. The corresponding values for 2a–e were in the range of 6.60–7.56 (HLB for 2a–e are 7.56, 6.60, 7.17, 6.99, and 6.99 respectively). Interestingly, HLB values of Boc-derivatives 3a–f were 9.90, 9.56, 8.08, 8.96, 8.68, and 8.68, respectively, which suggest that those

![Fig. 3](image1.png) SEM images of samples of 1a (A), 1b (B), 1c (C), 1d (D) 1e (E) and 2b (F) prepared by directly drop-casting their acetone solution (1 mg/1.5 mL) on a silica substrate.

![Fig. 4](image2.png) (A and B) TEM image of a sample of 1c prepared by directly drop-casting its acetone solution (1 mg/1.5 mL) on a carbon-coated copper grid.
with HLB between 8.0–10.0 have a higher tendency to give spherical aggregates. Critical Micellar Concentrations (cmc) of 1a–e were calculated using a fluorescence-based method using pyrene as the probe, and were found to be 0.9, 0.8, 1.0, 0.9 and 0.9 mM respectively. To gain a better understanding of molecular packing, the samples of 1a–e were subjected to PXRD analysis. They had peaks corresponding to d values in the ratio 1 : 1/2 : 1/3, suggesting layered assemblies. PXRD spectra of 1a, 1c and 1d are shown in Fig. 5 and those of others are given in the ESI† (Table SI-1).

As per the literature, a thin film-hydration method for drug-loading generally utilizes solvents like chloroform, dichloromethane or methanol for dissolution of components. 37-39 Drug delivery systems based on phosphatidylcholine, phosphatidylserine, and phosphatidyl ethanolamine require additives like cholesterol, span or tween for better stability, elasticity and permeability. 40 Since the compounds 1b–e were independently able to form spherical assemblies, they seemed to offer some advantage. When solutions of 1c in CHCl₃ or water were examined, short ribbon like aggregates with small twists were the result. In THF, it formed spherical aggregates (Fig. SI-9, ESI†). Based on these general observations, methanol was selected for dissolution of the components during the thin film hydration method in our preliminary studies.

Based on favourable aggregation characteristics of 1a–e, they were taken forward for detailed drug loading and release studies using ibuprofen as the model drug. Though effective as a NSAID, its low aqueous solubility and short plasma half-life (∼1–3 h) are the major limitations which can be addressed through improved formulation strategies. 41 The thin film hydration method was used to prepare their formulations. In this, a methanol solution of ibuprofen, and the lipid was subjected to rotary evaporation under reduced pressure at 40 °C to obtain a thin film. It was hydrated using phosphate buffer (pH 7.2) to get a suspension and then sonicated for 30 min to get more-or-less uniformly sized particles. Another set of samples containing cholesterol as the additive was also prepared for comparison of properties. All these formulations contained the amphiphile, drug and cholesterol in the ratio 1 : 1 : 0.5. These formulations are labelled as NC1a–e or N1a–e to indicate the constituents where ‘N’ represents ‘Non-ionic amphiphile’ and ‘C’ represents ‘Cholesterol’ (Table SI-2, ESI†). For example, NC1c represents the aggregate from 1c containing the drug and cholesterol, whereas N1c represents the same system without cholesterol. The standard used was liposomes from phosphotidylcholine and cholesterol, and is labeled as LCPC.

To get uniform size distribution after drug loading, the samples were extruded five times through a cellulose membrane (pore size 1000 nm) before analysis using SEM, TEM, DLS and qNano. From the SEM analysis, the diameters of aggregates containing cholesterol were estimated to be in the range of 40–500 nm (Fig. SI-10, ESI†). Without cholesterol, the sizes of aggregates were relatively larger and had diameters between 200 and 600 nm (Fig. SI-11, ESI†). Aggregates from NC1c are shown in Fig. 6A as a representative example. Its TEM image indicated a vesicular structure with an outer layer thickness of ∼11 nm as shown in Fig. 6B. Size distributions after passing 40 µL each of NC1c solution through nanopores of 1000 nm (NP1000), 800 nm (NP800) and 300 nm (NP300) were analyzed using qNano and the results are shown in Fig. 6C. As can be seen, the use of NP300 reveals the presence of smaller particles in the range of 100–200 nm. In comparison, DLS results suggest the particle diameter to be in the range of 400–600 nm (Fig. 6D) which represents the hydrodynamic diameter (Fig. SI-11, ESI†).

To know the nature of aggregates in their native conditions, CTEM analysis of samples of 1c and NC1c was performed. 42,43 As shown in Fig. 7A, the image of samples from 1c showed clear circular boundary indicative of a vesicular structure. However, the aggregates of NC1c (Fig. 7B), although spherical, looked slightly different. They appeared spherical with a denser interior. Such aggregates have been reported previously for drug-entrapped liposomal and niosomal systems. 44 The presence of cholesterol led to clustering of vesicles which is also evident in Fig. 7B (also in Fig. SI-12, ESI†).
The entrapment efficiency of NC1a–e and N1a–e, estimated based on the change in absorbance of the supernatant during thin-film hydration, is presented in Table 1. As can be seen, the efficiency of these formulations varied from 27–66%. There was a clear advantage of using cholesterol in the formulation which becomes clear if we compare the efficiencies of NC1a–e vs. N1a–e. The maximum efficiency (66%) is shown by the formulation based on the Phe-containing amphiphile NC1c.π-Stacking between the phenyl group in the drug and the amino acid spacer, along with H-bonding interactions as shown in Fig. 8, could be assisting drug-entrapment in this case. Upon adding ibuprofen to a solution of 1c in CDCl3, there was a down-field shift of an amide NH signal and up-field shift of an aromatic signal of 1c. This was accompanied by an up-field shift in the carboxyl carbon (13C NMR) of ibuprofen which collectively supports this assumption. These details are presented in the ESI.

Those with iPr and iBu side chains (NC1d and NC1e) were next in the line whereas Gly- and Ala-containing amphiphiles (NC1a and NC1b) were less efficient. These suggest that the amino acid residue connecting the head-group and the alkyl chain can control the aggregation and drug entrapment, making this design a modular one. To know the loading content, the drug-loaded aggregates (NC1a–e and N1a–e) were treated with 0.5% TritonX-100 and diluted to 1 mL using phosphate buffer (pH 7.2). After centrifugation, the absorbance of the supernatant was noted, from which the concentration of entrapped ibuprofen was calculated using the standard calibration curve (Table SI-5, ESI†). The values were comparatively less compared to those shown in Table 1 (likely due to incomplete disruption of aggregates in the presence of TritonX-100), NC1c–NC1e remained superior, with loading contents in the range of 34.52–43.42%. The stability of these aggregates was subsequently assessed by noting the morphology and loading content after storing the suspensions for 12 days (Fig. SI-14 and Table SI-6, ESI†). The morphology was found to remain more or less intact upon simple exposure to aqueous buffer whereas TritonX-100 treatment induced some deformation. There was only very little reduction in loading content after storage which is also noteworthy. Many soyaphosphatidylcholine-based drug formulations are in clinical use (ref. 8) and the fact that the difference in the entrapment efficiency of NC1c compared to this standard is only 12% giving hope that oxanorbornane-based amphiphiles can be developed into a new class of drug delivery vehicles. Like drug-entrapment, drug-release is also an important property that will determine the success of drug formulations. Drug release was monitored using a dialysis technique for a period of 24 h. Initial burst release was not observed in any of the formulations. A plot of percentage drug release vs. time, shown in Fig. 9, allows us to make a comparison of the drug release profiles of these systems with that based on soyaphosphatidylcholine. At the end of 24 h, 45% ibuprofen release was observed for NC1c while 49% was observed for liposome formulation based on soyaphosphatidylcholine.

Release from Leu- and Val-based formulations (NC1e, NC1d) were 24% and 35% respectively (Fig. 9 and Table SI-4, ESI†). Since drug release from such systems happens through diffusion, the rate difference seen in these formulations could be due to the difference in molecular packing and the extent of solvent interaction. To gain some insight into the distribution of small

Table 1

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*Composition of formulations and experimental data are given in the ESI (Table SI-2).
molecules in these aggregates, a confocal imaging study was performed using 1c loaded with fluorescein. The cross-sectional images of one of the aggregates showed that the dye is uniformly present throughout as shown in Fig. 10. In comparison with the image shown in Fig. 7a (vide supra), the aggregate shown here has a larger size, and is likely formed through coalescence of several smaller aggregates (diameter ~6 μm). This clearly shows the ability of these lipids to entrap small molecules and also indicates that the nature of the aggregates could vary from vesicles to SLPs (solid–lipid particles) depending upon the composition and other parameters related to sample preparation.

**Conclusion**

A modular approach to new drug delivery vehicles based on non-ionic amphiphiles with a hydroxylated oxanorbornane head group is presented. Although their self-assembly is primarily driven by head group interactions and alkyl chain clustering, it was possible to fine-tune the aggregation using an amino acid residue as the spacer. Conjugates with a C7 alkyl chain with intervening Phe-, Val- or Leu-residues showed a high tendency to form spherical aggregates and were subjected to detailed drug-loading and -release studies both in the presence and absence of cholesterol – a commonly used additive in such formulations. Ibuprofen, a well-known NSAID, was used as the model drug in these experiments. An entrapment efficiency of 66% (cf. soyosphatidylcholine based formulation 78.6%) and 45% drug release at the end of 24 h was observed in the case of the Phe-containing amphiphile. Apart from maintaining the spherical nature of aggregates, the amino acid residue in such systems appears to assist drug-entrapment as well.

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