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# Novel agrochemical conjugates with self-assembling behaviour

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**ABSTRACT**

Hypothesis: That conjugation of agrochemicals to pro-assembly hydrophobic moieties will enable enhanced compatibility and loading with host lyotropic liquid crystalline carrier matrix, and potentially self-assemble in their own right in aqueous environments. Experiments: A series of lipid-like agrochemical-conjugates were synthesized using specific amphiphilic entities conjugated onto the agrochemicals, picloram and 2,4-dichlorophenoxyacetic acid (2,4-D). The self-assembly behavior and compatibility of the novel entities when incorporated into phytantriol and monoolein-based liquid crystalline systems were examined using small angle X-ray scattering, cryo-TEM and polarized optical microscopy. Findings: Compared to agrochemical-conjugates with simple alkyl ester groups, the esterification of the agrochemicals with amphiphilic groups such as phytantriol and monoolein led to greater structural compatibility and consequently a greater loading of the agrochemicals in the liquid crystalline systems without destabilizing phase structure. Picloram-monoolein and picloram-monoelaidin can self-assemble to form lamellar structures in water. However, certain agrochemical-conjugates such as picloram-monoelaidin and picloram-PEGn-oleate showed poor compatibility with liquid crystalline systems, resulting in phase separation.

## 1. Introduction

Liquid crystalline structures such as the inverse bicontinuous cubic and inverse hexagonal phases can be formed when certain polar lipids such as phytantriol, glyceryl monooleate (GMO) and glyceryl monoelaidin (GME) are exposed to excess water. These lipid-based liquid crystalline (LC) systems are of interest as potential drug delivery agents due to the possibility to disperse them as internally structured emulsions, their ability to encapsulate hydrophilic and lipophilic actives in the aqueous and lipid-rich regions, respectively, and impart controlled release properties [1-3].

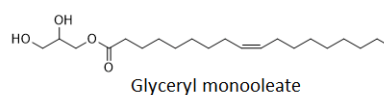
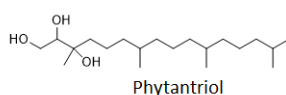
Recent studies have shown that plants exposed to liquid crystalline particles with the internal inverse cubic structure exhibited significantly less phyto-toxicity compared to those exposed to traditional surfactant-based adjuvant systems [4]. The LC systems also showed significant penetration of the encapsulated agents into the leaf, in addition to potential benefits in increased rain-fastness and controlled release, all of which leads to increased active efficiency while reducing environmental impacts due to run off [5]. However, the potential use of lipid-based liquid crystalline systems as agrochemical delivery agents is limited by low encapsulating capacity. Many of the agrochemical actives are “amphiphobic” and cannot be encapsulated in either the aqueous or lipophilic regions of the LC systems at application relevant concentrations [6-8].

Alkylation of the agrochemical actives is a common strategy used in the industry to improve the compatibility of the active agrochemical with commercial oil-in-water emulsion formulations [9]. Similarly, the alkylation of actives would also allow greater compatibility between the agrochemical and the lipid-rich regions of the LC systems. However, previous studies had shown that the addition of hydrophobic substances such as vitamin E acetate, alkanes and fatty acids at low pH into LC systems can disrupt the critical packing arrangement of the lipids resulting in the loss of ordered structures [4, 10-12]. As such, it was hypothesized that the alkyl-conjugates which contain the agrochemical active and a lipophilic tail, but no significant hydrophilic component, when added to LC systems, will induce structural instability similar to that of typical hydrophobic additives and consequently, loss of any structural-based benefits that LC systems may have as agrochemical delivery agents.

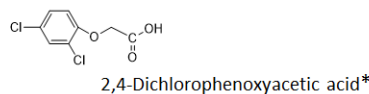
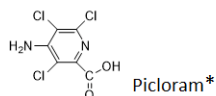
In this study, novel agrochemical-conjugates were synthesized where the actives, 2,4D acid and picloram, were covalently bonded to amphiphilic entities such as glyceryl monooleate, phytantriol, PEG-oleate and glyceryl monoelaidin to promote incorporation into lyotropic liquid crystals. Their structural impact when loaded into phytantriol and GMO-based inverse cubic liquid crystalline systems, was examined and compared with simple alkyl-conjugates. The chemical structure of 2,4-D, picloram and their lipid-conjugates; and phytantriol and GMO are shown in Fig. 1.

It was hypothesized that: i) the bonding of amphiphilic molecules instead of simple alkyl chain on the amphiphobic agrochemicals will allow better structural compatibility with the LC systems and consequently higher loading capacity; ii) these lipid-conjugates themselves will self-assemble in water and may intrinsically form LC structures without the need of lipid-based LC systems as scaffolds.

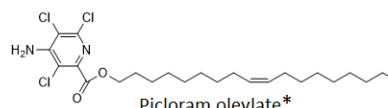
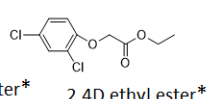
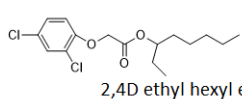
#### LC forming lipids



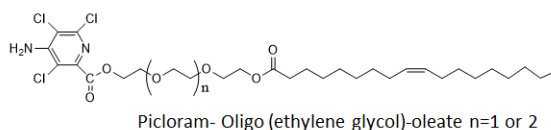
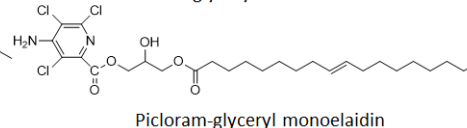
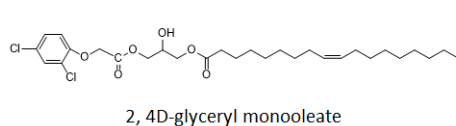
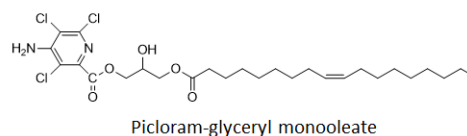
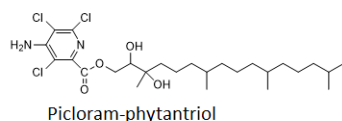
#### Agrochemical active



#### Alkylated agrochemical active



#### Amphiphilic agrochemical actives



**Fig. 1.** The chemical structures of agrochemicals and liquid crystalline forming lipids. \*denoted commercially available conjugates used in this study.

## 2. Experimental and Methods

### 2.1. Materials

Phytantriol was kindly donated by Roche (Grenzach-Wyhlen, Germany) with a nominal purity of >96.6%. Glycerol monooleate (GMO) (in the form of Myverol 18-99K) was kindly donated by Kerry Scientific (Norwich, NY), and had a GMO content of 60.9% w/w. Glycerol monoelaidin (GME) (purity >97.0%) was purchased from TCI Co. Ltd. (Tokyo, Japan). Pluronic F127 (analytical grade) was purchased from BASF (Somerset, NJ). Picloram (PIC) and 2,4-dichlorophenoxyacetic acid (2,4-D), picloram oleylate (PIC-Oleyl), 2,4D ethyl ester and 2,4D ethylhexyl ester were kindly donated by Nufarm Co. Ltd. (Victoria, Australia). Poly(ethylene glycol) monooleate average  $M_n \sim 460$  (PEG-oleate), O-(6-chlorobenzotriazolyl)-N,N',N'-tetramethyluronium hexafluorophosphate (HCTU) (purity >98.0%) and triisopropylamine (purity >98.0%) were purchased from Sigma-Aldrich (St. Louis, MO). Monodispersed oligo(ethylene glycol) monooleates were obtained via further chromatography isolation using diethyl ester as eluent. Diethyl ether, chloroform, methanol, acetonitrile and magnesium sulfate anhydrous were purchased from Merck Pty. Ltd. (Kilsyth, VIC, Australia).

### 2.2. Synthesis of the agrochemical-conjugates

The novel entities were synthesized using the following procedure: 10 mmol of picloram or 2,4-D acid, was suspended in 150 mL acetonitrile in a round-bottom flask equipped with a magnetic bar, HCTU (12 mmol) was added into the round-bottom flask under magnetic stirring. After 10 min of stirring, 20 mmol of phytantriol or GMO dissolved in acetonitrile (20 mL) was added followed by another 10 min of stirring and then 30 mmol of triisopropylamine (4.3 mL) was added. The mixture was left stirring overnight at room temperature to ensure complete reaction.

The solution was dried using a rotary evaporator under vacuum and then extracted by using 50 mL of diethyl ether three times. The extracted solutions of diethyl ether were combined and vacuum dried to obtain the crude product. The crude product was purified further using gravity

chromatograph and chloroform as eluent. Typical yield was > 50 %. The purity and chemical structure of the synthesized compounds were confirmed using  $^1\text{H}$  NMR (See Supplementary Information).

### 2.3. Preparation of agrochemical loaded-LC systems

The synthesized agrochemical-conjugates and lipid were first dissolved in ethanol in order to create individual stock solutions with concentration of 100 mg/mL. The stock solutions were mixed at appropriate volume ratio in a transparent 96 well plate (PerkinElmer), up to total volume of 200  $\mu\text{L}$ . After loading, the well plates were agitated on a platform mixer for 3 h, and ethanol was then removed under vacuum, resulting in a thin lipid film ( $\sim 20$  mg) at the bottom of the wells [13].

For bulk non-dispersed samples, 50  $\mu\text{L}$  of Milli-Q water was added into the wells to hydrate the lipids. The plate was immediately film sealed to prevent evaporation and subjected to further agitation on the plate mixer overnight at 40  $^{\circ}\text{C}$  and then stored at 25  $^{\circ}\text{C}$  for over 24 h for equilibration [14].

For preparation of dispersed samples, 200  $\mu\text{L}$  of 1.5 % w/w F127 solution was added to the dried lipid film in the wells and the well plates were then film sealed and carefully placed in a sonication bath. The plates were subjected to sonication for over 30 min and resulted in the formation of opaque dispersions.

### 2.4. Small angle X-ray scattering (SAXS)

SAXS measurements were conducted on the SAXS/WAXS beamline at the Australian Synchrotron (Victoria, Australia)[15] to identify the internal structure of the dispersed and bulk non-dispersed systems for the various lipid and agrochemical-conjugates. The transparent 96-well plates loaded with samples were mounted vertically in the beam path and scans were automated using a pre-loaded set of position variables based on the well positions within the plate, the exposure times were 1 sec. The 2D scattering patterns were collected using a Pilatus 1 M detector and radially integrated using the in-house software “ScatterBrain”. The experiments used a beam of wavelength  $\lambda = 1.13$  Å (11.0 keV), sample to detector length of approximately 0.9 m, and a typical flux of  $1.2 \times 10^{13}$  photons/s. The liquid crystal phase structures were determined by indexing the Bragg peaks according to their corresponding reflection laws [16].

### 2.5. Polarized optical microscopy (POM)

A Nikon DS-Fi2 microscope fitted with crossed polarizing filters and at 100-fold magnification, was used to identify the internal structure of bulk non-dispersed systems to complement the SAXS data. A small amount of the dried lipid+agrochemical-conjugate mixture from the well plates was wedged between a microscope slide and a cover slip, and the sample was then flooded with excess water. The texture, birefringence and viscosity of the hydrated mixture was analyzed to identify the LC structure [17].

### 2.6. Cryogenic transmission electron microscopy (cryo-TEM)

Cryo-TEM imaging was used to study the morphology of dispersed systems and was conducted at CSIRO (Parkville, VIC, Australia). The preparation procedure of samples for imaging has been described previously [18]. Briefly, a drop of dispersion sample was placed on a TEM grid blotted with filter paper, and then the grid with sample was rapidly plunged into liquid ethane. The sample was stored under liquid nitrogen and transferred to a TEM (FEI Tecnai 20) operating at 120 kV for imaging while maintaining the sample at  $-170\text{ }^{\circ}\text{C}$  [18].

## 3. Results and discussion

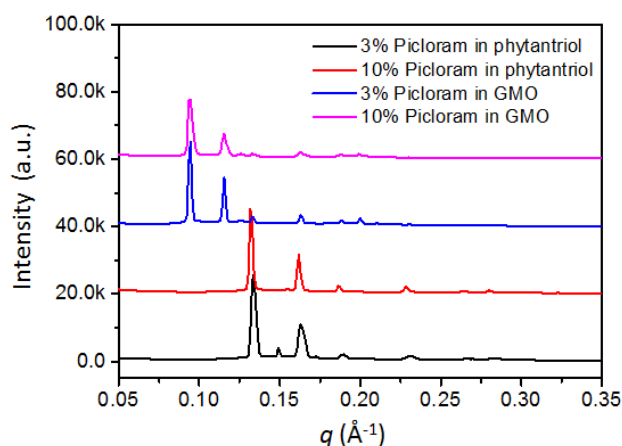
### 3.1. Impact of picloram and picloram-conjugates on the lyotropic phase behavior of phytantriol and GMO-based bulk LC systems

During the mixing of picloram with GMO and phytantriol, it was observed that the maximum solubility of picloram in lipids only allowed loading up to 3 % w/w (equal to 4 % mol/mol to phytantriol or 4.3 % mol/mol to GMO). Higher concentrations of picloram in lipid resulted in significant coexisting solid picloram. The SAXS profiles of picloram-loaded phytantriol and GMO-based LC bulk phase in excess water are shown in Fig. 2. Phytantriol and GMO form the inverse cubic phase with the Pn3m space group ( $V_{2(\text{Pn3m})}$ ) in excess water at ambient temperature [19].

The  $V_{2(\text{Pn3m})}$  phase of phytantriol and GMO-based systems was retained at picloram concentration of 3 % w/w. A further increase in the picloram concentration to 10 % w/w (equal to 13.2 % mol/mol to phytantriol or 14 % mol/mol to GMO) had no impact on the relative



position of the Bragg peaks, indicating that picloram had minimal effect on LC structures even when present with solid picloram in excess.



**Fig. 2.** SAXS profiles of phytantriol and GMO-based bulk LC systems loaded with varying weight concentration of picloram, in excess water, at ambient temperature.

### 3.2. Effect of picloram-conjugates on phytantriol bulk cubic phase

The SAXS profiles of the phytantriol-based bulk LC systems with increasing molar concentration of the picloram-conjugates are shown in Fig. 3. The lattice parameters of the identified structures are summarized directly below the corresponding SAXS profiles. The shift in Bragg peaks to higher values of  $q$ , indicating smaller lattice dimensions, with increasing concentration of the oleyl-modified picloram-conjugate (PIC-Oleyl) suggests the compound increases the negative curvature of the lipid packing in the system (Fig. 3A and 3B). At above 3.3 % mol/mol in phytantriol, PIC-Oleyl induced a phase transition from the  $V_{2(\text{Pn3m})}$  phase to inverse hexagonal ( $H_2$ ) phase, and then to the inverse micellar phase ( $L_2$ ) at above 14 % mol/mol. The structural impact of PIC-Oleyl on phytantriol-based LC system is similar to that of typical hydrophobic materials [10, 20].

In contrast to PIC-oleyl, higher concentrations of picloram-conjugates with amphiphilic functional groups could be loaded into the LC system at greater levels without inducing a phase transition. The addition of PIC-phyt in the phytantriol-based LC system induced a decrease of the lattice parameter for the  $V_{2(\text{Pn3m})}$  phase but only the  $V_{2(\text{Pn3m})}$  phase was observed up to 7.8 %

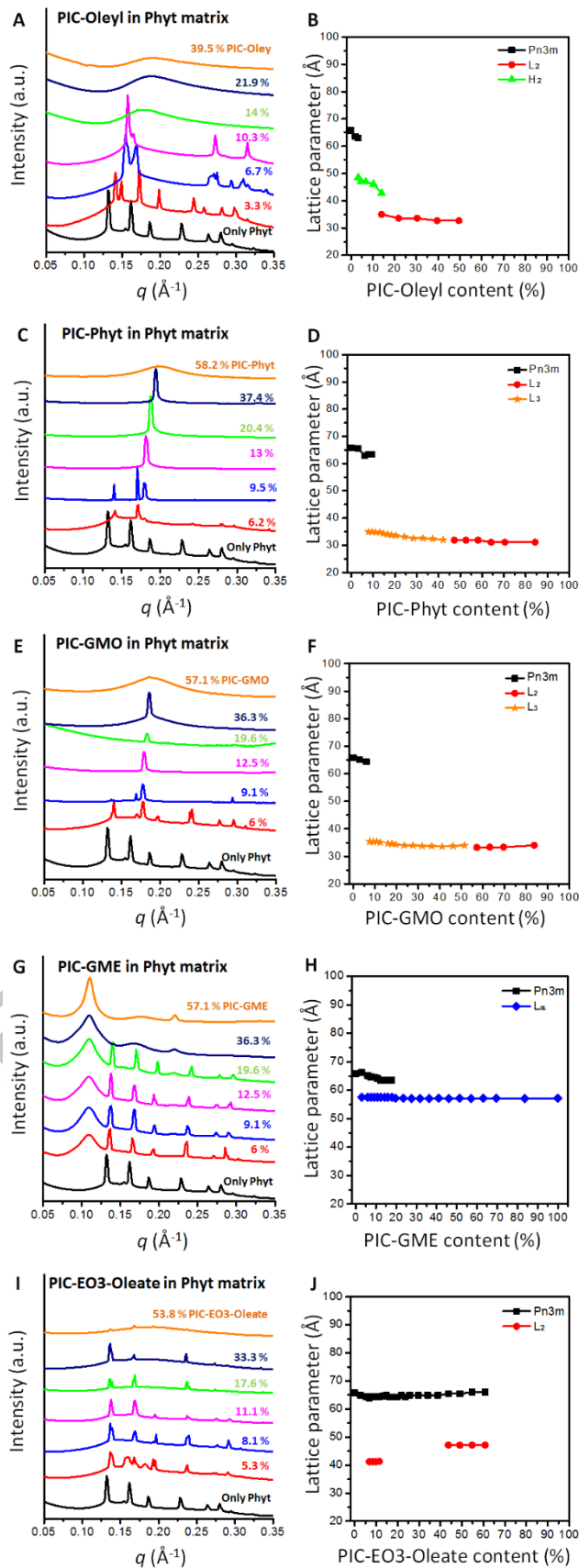
mol/mol of PIC-Phyt in phytantriol. At concentration of 7.8-12.2 % mol/mol in phytantriol, a mixture of  $V_{2(Pn3m)}$  phase and a second phase represented by a single sharp peak between ( $q = 0.15 - 0.2 \text{ \AA}^{-1}$ ) was observed. The second phase is most likely an  $L_3$  sponge phase, and the single sharp peak is likely from bilayer correlations from the  $L_3$  sponge phase based on previous literature [20, 21]. While this assignment cannot be uniquely made on the basis of the single peak, the greater correlation compared to the very broad peak indicative of  $L_2$  phase, and lack of a second reflection indicating lamellar phase leads us to this putative assignment. At above 12.2 % mol/mol, the  $V_{2(Pn3m)}$  phase was lost and only the  $L_3$  phase is apparent which ‘melts’ to the  $L_2$  inverse micellar phase above approximately 50 % mol/mol.

Only the  $V_{2(Pn3m)}$  phase was observed at up to 6 % mol/mol PIC-GMO in phytantriol, with progressive decrease in lattice size with increasing PIC-GMO concentration. At above 7.9 % mol/mol and up to 51.5 % mol/mol, again it is likely that an  $L_3$  sponge phase was observed, which “melts” to the  $L_2$  inverse micellar phase on addition of PIC-GMO above approximately 55 % mol/mol.

The  $V_{2(Pn3m)}$  phase was retained at up to 31.8 % mol/mol PIC-GME in phytantriol, with progressive decrease in lattice dimension with increasing PIC-GME concentration, similar to the impact of PIC-Phyt and PIC-GMO. However, there was also a broad peak at  $q \sim 0.11 \text{ \AA}^{-1}$  present at 3 % mol/mol PIC-GME to phytantriol, with concentration independent  $q$  value, which persisted up to 100 % PIC-GME. Furthermore, the scattering intensity of the singular peak appeared to increase with increasing PIC-GME concentration. This suggests that although PIC-GME does incorporate and interact with the phytantriol-based LC system, there is significant phase separation. At higher concentrations of PIC-GME two distinctive Bragg peaks at  $q \sim 0.11$  and  $0.22 \text{ \AA}^{-1}$  are more evident in the scattering profile indicating that PIC-GME self-assembles to form the lamellar structure in water.

The constant position of the Bragg peaks with increasing concentration of PIC-EO3-Oleate to phytantriol suggests that this conjugate does not incorporate into the lipid domains of the inverse cubic system. The appearance of a diffuse scattering peak between  $q \sim 0.15 - 0.20 \text{ \AA}^{-1}$  with increasing scattering intensity with increasing concentration of the agrochemical-conjugate also suggests the phase separation between phytantriol and PIC-EO3-Oleate, and that the compound may form inverse micellar phase in water.

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**Fig. 3.** The SAXS profiles and corresponding lattice parameters of phytantriol-based bulk non-dispersed systems with increasing molar concentration of PIC-Oleyl (A and B), PIC-Phyt (C and D), PIC-GMO (E and F), PIC-GME (G and H) and PIC-EO3-Oleate (I and J). For clarity, only selected scattering profiles are shown, but all calculated lattice parameters are illustrated.

### 3.3. Effect of picloram-conjugates on GMO bulk cubic phase

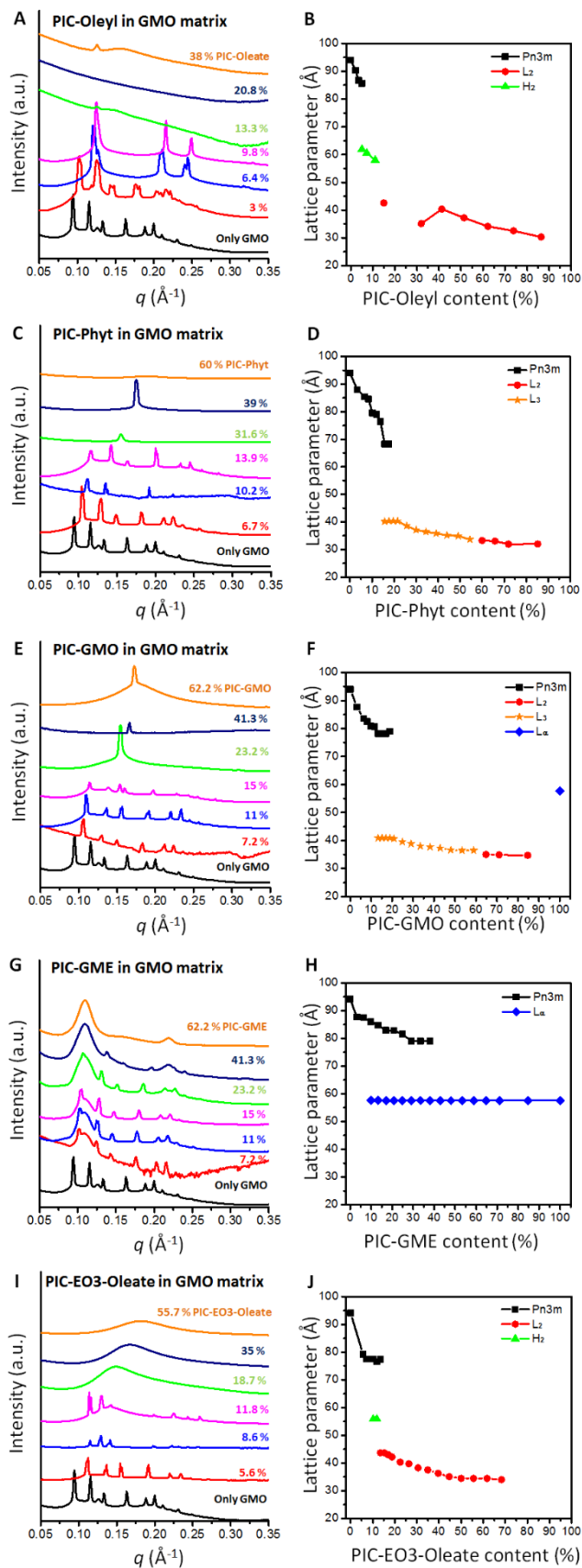
The SAXS profiles of GMO-based bulk LC systems with increasing concentration of picloram-conjugates are summarized in Fig. 4. In summary, GMO containing PIC-Oleyl transitioned through the inverse hexagonal ( $H_2$ ) phase to the inverse micellar phase ( $L_2$ ) with increasing concentration of PIC-oleyl (Fig. 4A and 4B). Addition of a greater proportion of PIC-Phyt was required to transition the GMO matrix to the inverse micellar phase ( $L_2$ ) via a putative  $L_3$  sponge phase with no intermediate  $H_2$  phase being observed (Fig. 4C and 4D) and was essentially the same as addition of PIC-GMO to the GMO system (Fig. 4E and 4F).

Addition of PIC-GME at increasing concentration to the GMO bulk cubic phase induced a decrease in the lattice parameter of the  $V_{2(Pn3m)}$  phase (Fig. 4G and 4H). This indicated that the PIC-GME is more compatible with the GMO-based LC system and resides in the lipid bilayers, in contrast to the PIC-GME+phytantriol LC system where phase separation was observed at very low loading concentrations. The  $V_{2(Pn3m)}$  phase was retained up to 38 % mol/mol PIC-GME to GMO. However, the Bragg peaks indicating lamellar structures formed by the self-assembly of PIC-GME in water were also observed at above 9.8 % mol/mol PIC-GME to GMO, compared to at above 3 % mol/mol in the phytantriol system. The phase separation of PIC-GME from GMO-based cubic phase was also observed using polarized optical microscopy where the mixture in excess water showed the birefringent cross pattern representing the lamellar phase coexisting with viscous non-birefringent patterns typical of the inverse cubic phase (Fig. 5).

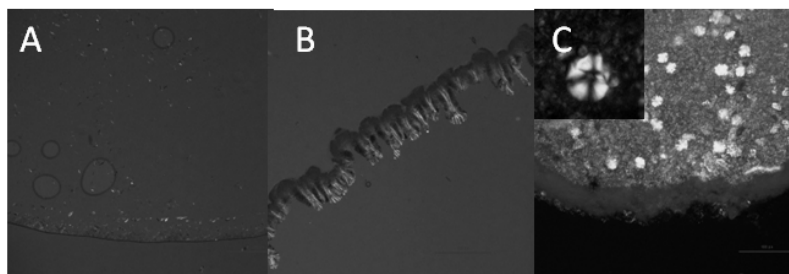
Similar to the PIC-GME case, PIC-EO3-Oleate integrated more readily into the GMO cubic phase (Fig. 4I and 4J), in contrast to the phase separation observed in the phytantriol-based system. Only the  $V_{2(Pn3m)}$  phase was observed up to 8.6 % mol/mol PIC-EO3-Oleate to GMO. The  $H_2$  and the  $L_2$  phases were observed at 10.2-11.8 % mol/mol and 13.5-15.2 % mol/mol to GMO, respectively, which co-existed with the  $V_{2(Pn3m)}$  phase. At above 15.2 % mol/mol, only the  $L_2$  phase was observed. Thus the general trend in phase behaviour for this group of conjugates is towards more negatively curved packing with increased conjugate concentration, evidenced by

the decreasing lattice parameter, and presence of intermediate inverse hexagonal phase for some of the conjugates.

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**Fig. 4.** The SAXS profiles and corresponding lattice parameters of GMO-based bulk lyotropic systems with increasing molar concentration of PIC-Oleyl (A and B), PIC-Phyt (C and D), PIC-GMO (E and F), PIC-GME (G and H) and PIC-EO3-Oleate (I and J). For clarity, only selected scattering profiles are shown, but all calculated lattice parameters are illustrated.



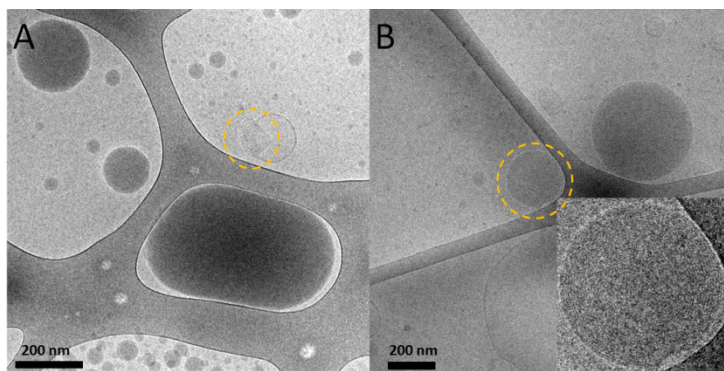
**Fig. 5.** The polarized optical images of PIC-GME+GMO bulk phase in excess water. The PIC-GME contents in GMO are 20.8 % (A), 38 % (B) and 65 % (C) mol/mol. Insert highlights a magnified section displaying a maltese cross indicative of lamellar phase being present.

### 3.4. Dispersed particle systems

The structures of phytantriol and GMO dispersions containing the picloram-conjugates were very similar to the equivalent bulk LC systems (Fig. 12S and 14S), with some slight shifts in the concentration-dependent phase transition boundaries being apparent. The bulk phases have different water content to the dispersion systems and it is possible for the picloram-conjugates to partition between the excess water phase and the LC phase which could lead to differences in phase behaviour. For example, in the PIC-EO3-Oleate+GMO system the  $H_2$  phase was observed in the bulk system but not in the equivalent dispersion system. Alternatively, the absence of  $H_2$  phase in equivalent dispersion compared to bulk phase has been previously observed in pure phytantriol-based systems [10] and was attributed to difference in packing frustration in the dispersed particles with limited repeating units compared to the bulk [22].

The phase separation observed for phytantriol and GMO systems containing PIC-EO3-Oleate and PIC-GME, respectively using SAXS, was also apparent in cryo-TEM where co-existence of cubosomes and other particles was apparent (Fig. 6).





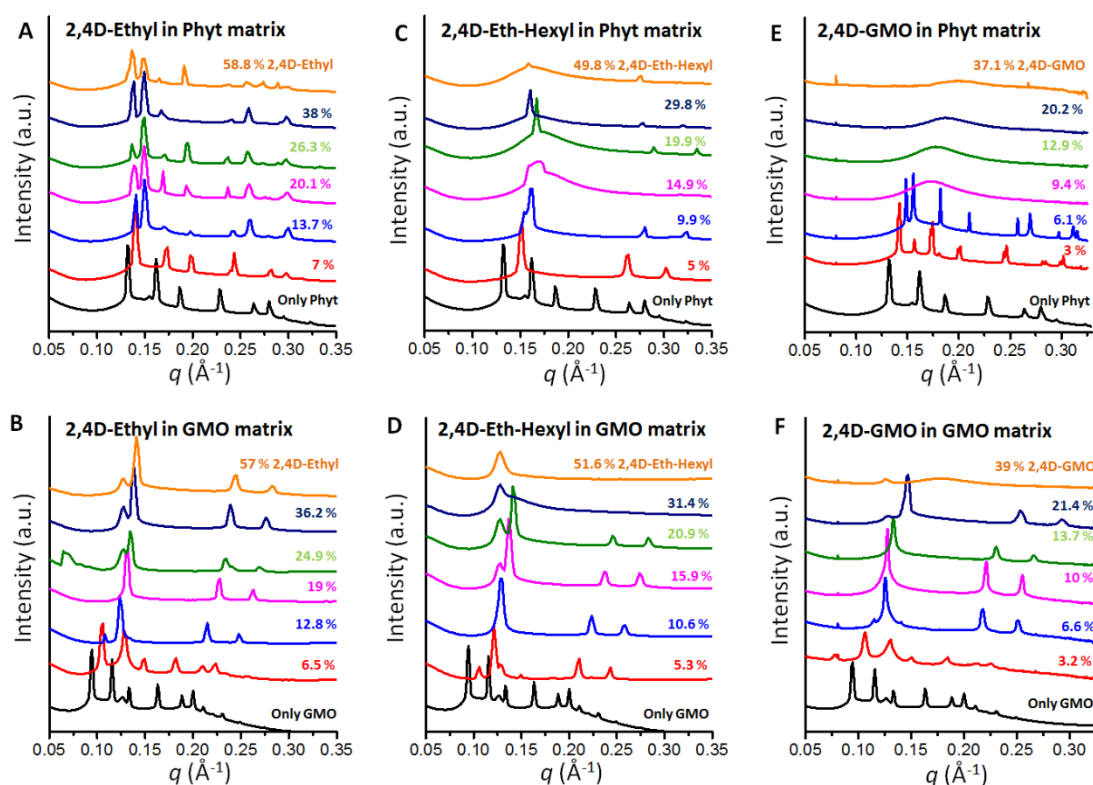
**Fig. 6.** Cryo-TEM images of dispersions containing 53.8 % mol/mol PIC-EO3-Oleate in phytantriol (A) and 24.9 % mol/mol PIC-GME in GMO (B). In Panel A, the circle is indicating a square faceted co-existing cubosome particle, while in Panel B the circle indicates a multilamellar particle.

### 3.5. Impact of 2,4D-conjugates on structure in lyotropic liquid crystalline systems

The loading of 2,4D acid into GMO and phytantriol-based systems was low (3% w/w; equal 4.1 % mol/mol in phytantriol, or 4.7 % mol/mol in GMO) with crystalline material being evident at higher loadings, similar to the case of picloram.

The SAXS profiles of phytantriol and GMO-based bulk LC phases with increasing concentration of 2,4D conjugates are summarized in Fig. 7. The addition of commercial 2,4D ethyl ester induced a phase transition from the inverse cubic phase to the inverse hexagonal phase at above 9.7 % mol/mol and 10.3 % mol/mol for phytantriol and GMO systems (Fig. 7A and 7B), respectively. Apparent coexisting hexagonal and  $L_2$  inverse micellar phases occurred at higher levels of the 2,4D ethyl ester. The longer alkyl chain of the commercial ethyl hexyl ester induced the inverse cubic to inverse hexagonal phase transitions at above 3.9 % mol/mol and 3.2 % mol/mol for phytantriol and GMO systems (Fig. 7C and 7D), respectively. At higher concentrations (>16% mol/mol), the alkyl-conjugates also induced the inverse hexagonal to inverse micellar phase transition.

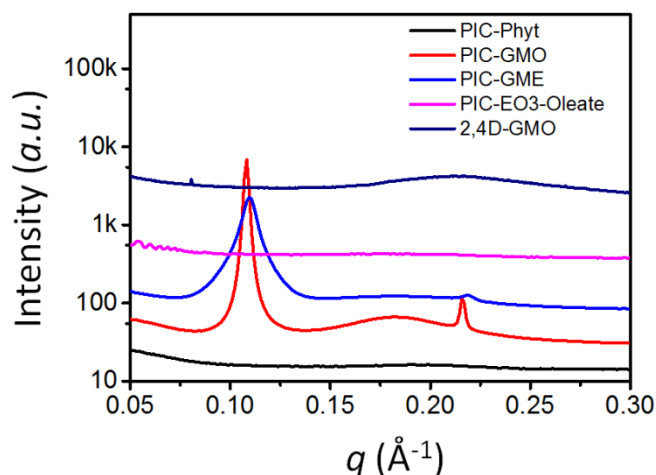
The novel 2,4D-GMO conjugate, despite having a longer unsaturated alkyl chain (C18:1) was not particularly compatible with the matrix, inducing the inverse cubic to inverse hexagonal phase transition at above 5% mol/mol, and inverse hexagonal to inversed micellar phase transition at above 9.4% mol/mol and 33% mol/mol for phytantriol and GMO systems (Fig. 7E and 7F), respectively.



**Fig. 7.** The SAXS profiles of 2,4D-ethyl ester (A and B), 2,4D-ethyl hexyl ester (C and D) and 2,4D-GMO (E and F) in phytantriol-based and GMO-based LLCs in bulk phase. The concentration of 2,4D-conjugates is the molar concentration relative to the core lipid.

### 3.6. Self-assembly of agrochemical-conjugates in absence of core lipid

The SAXS profiles of the amphiphilic conjugates-only in excess water are summarized in Fig. 8. Bragg peaks were absent for PIC-Phyt, PIC-EO3-Oleate and 2,4D-GMO indicated that they did not self-assemble into any ordered liquid crystalline structure. In contrast, both PIC-GMO and PIC-GME showed two Bragg peaks with vector ratio of 1:2, indicating the formation of a lamellar phase.



**Fig. 8.** The SAXS profiles of the pure amphiphilic agrochemical-conjugates dispersed in water

### 3.7. Discussion of the effect to LLC structures from agrochemical-conjugates additive

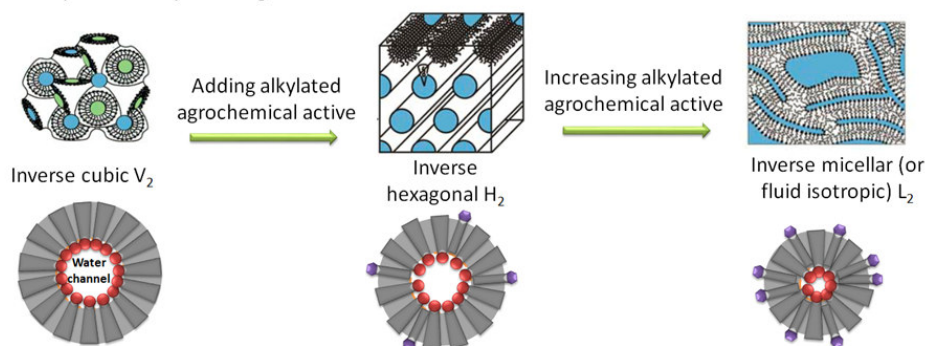
The addition of solvent compatible functional groups to a solvent-incompatible active in order to enhance solubility is a common strategy in both agrochemical and pharmaceutical fields. The alkylation of the agrochemicals generally allows for better active loading in the oil phase of surfactant-rich oil-in-water emulsion concentrates, which in turn, offer enhanced uptake of the actives when applied onto the waxy plant leaves [23, 24]. In such applications, the critical packing property of the alkylated agrochemical is not critical. Cubosomes and hexosomes are showing promise as agrochemical delivery systems due to their phase-dependent adhesive behavior and environmental compatibility. However, to enhance the loading of actives in the liquid crystalline systems without significant disruption to the internal nanostructure requires greater compatibility between the packing property of the agrochemical-conjugates and that of the LC-forming lipids. To the best of our knowledge, this is the first time that agrochemical-conjugates were rationally designed with aim of enhancing their compatibility and loading in LC systems.

Picloram and 2,4-D acid have an estimated partition coefficients ( $\log K_{o/w}$ ) of 1.9 and 2.8 [25], respectively, indicating a preference for the lipid phase, despite being poorly soluble in oil. As such, the conjugation of simple alkyl groups resulted in the formation of conjugates that are lipophilic. The incorporation of these conjugates into the lipid-based LC systems induced structural changes in a similar manner to that of typical hydrophobic additives as may be reasonably expected [10]. The lipophilic molecules reside deep in the lipid bilayers, which then

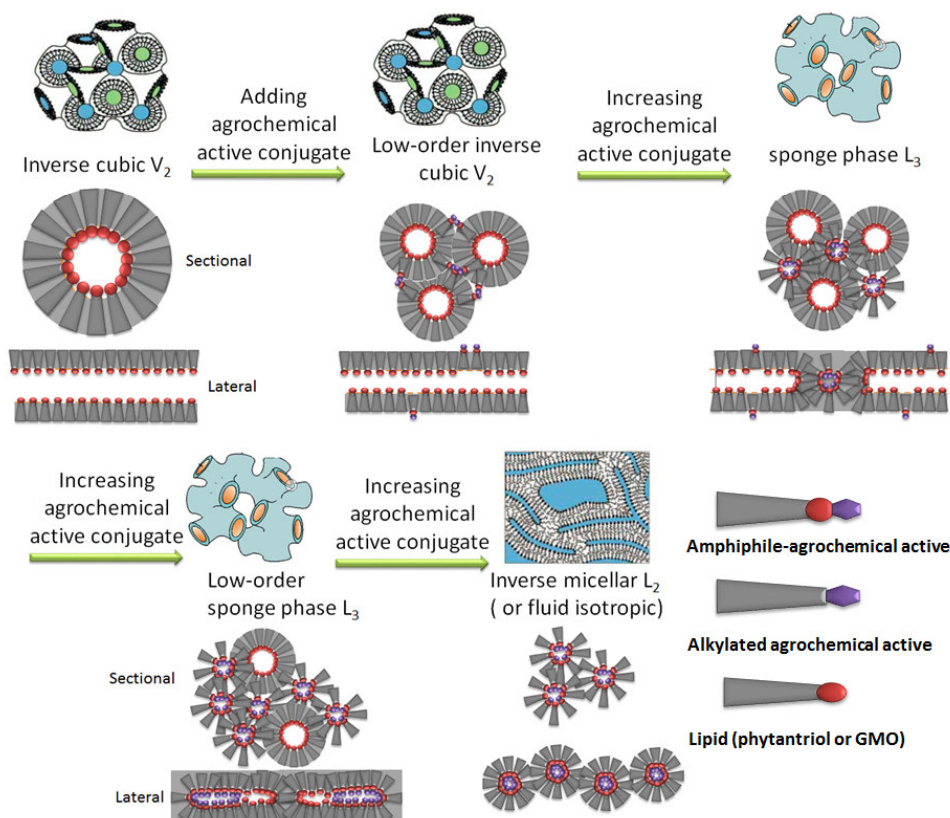
imparts a greater negative curvature effect and consequently leading a decrease in the lattice size of the inverse cubic phase at low concentrations, and phase change to the inverse hexagonal phase and then the inverse micellar phase at higher concentrations.

The amphiphile-picloram conjugates, which are more hydrophilic than the alkyl-active conjugates, were more compatible with the LC system, with greater loading possible without losing the inverse cubic structure. The possible mechanism of effect on LC structure based on compatibility of amphiphile-picloram with lipid was considered at the molecular and mesoscopic levels. At the molecular level, the amphiphile-picloram conjugates tend to form bilayer structures. The high compatibility of amphiphile-picloram with the host lipid phase allows them to co-assemble and retain the LC structure [26]. At higher concentrations, the packing is still disrupted by the presence of the guest amphiphilic molecule, but with reduced efficiency than the free agricultural chemical, leading to increasingly more negatively curved packing leading to deviation from the cubic phase to either inverted hexagonal phase, or disordered lamellar structures such as the putative  $L_3$  phase, or almost complete loss of order as the  $L_2$  inverse micellar phase. PIC-GME was the clear exception, forming a lamellar phase at high concentrations in the host matrix, and consistent with it self-assembling to form a lamellar phase for pure PIC-GME dispersed in water [27]. The proposed orientations of both the simple alkyl, and amphiphilic conjugates within the bilayer are illustrated in Fig. 9.

(A) The impact of alkylated agrochemical active to LLC



(B) The impact of amphiphile-agrochemical active conjugate to LLC



**Fig. 9.** The schematic of the possible orientations of alkylated-picloram and amphiphile-picloram conjugates in lipid-based LC systems and consequent impact on LC structures. Redrawn from Qiu, Bhansali & Caffrey [28-30].

In contrast to the amphiphile-picloram conjugates, 2,4D-GMO did induce the formation of the  $H_2$  phase at a wide range of concentrations in the LC systems (Fig. 7). It is hypothesized that this may be due to the greater lipophilicity of the 2,4-D acid headgroup compared to picloram, as

indicated by its higher  $\log K_{o/w}$ . The overall more hydrophobic compound then partitions deeper into the bilayer, acting in a similar manner to the simple alkyl conjugates.

To the best of our knowledge, this is the first time that agrochemical conjugates were designed to act as amphiphiles and to have designed-in compatibility with structured self-assembled carrier systems. The greater compatibility provides opportunity for enhanced loading into the liquid crystalline particulate carriers, which is commercially significant as it provides an opportunity to reduce the amount of carrier lipid required in the formulation. An additional aspect of these conjugates is that their enhanced lipophilicity may also provide an advantage in permeability through the lipid domains of the plant cuticle, potentially providing more effective delivery into the plant compared to the parent compound. Simple esters (for example the ethyl and ethyl hexyl derivatives of 2,4D studied here in addition to the new compounds) [31] are increasingly used for compatibility with carrier systems and these studies provide a guide to design of improved ester systems with specific affinity for lyotropic liquid crystal carrier systems.

#### 4. Conclusions

Previous work in loading agrochemicals into lyotropic liquid crystalline carriers was limited by the loading that could be achieved without a loss of structure of the host matrix [10]. Here we demonstrate an approach to overcome this problem by conjugating the agrochemical to a pro-assembly molecules to induce self-assembly of the conjugates themselves or to enhance their loading in the host lipid matrix. Novel picloram and 2,4D-ester conjugates were designed and synthesized with conjugation to GMO, phytantriol, monoelaidin, and PEG-oleate pro-assembly amphiphiles and compared to commercial ethyl ester and oleate systems. Their incorporation into host GMO and phytantriol cubic phases was then studied primarily using SAXS.

The GMO and PHYT conjugates allowed for higher quantity of actives to be loaded into the GMO and phytantriol-based inverse cubic systems before inducing phase changes, compared to the alkyl ester conjugates and free active [32, 33]. The addition of PIC-GME and PIC-PEGn-oleate to lipid-based LC systems resulted in phase separation indicating low compatibility and encapsulation. The picloram-GMO and picloram-GME conjugates also self-assembled in their own right to form the lamellar phase in excess water.



The concept of conjugating amphiphilic constructs on actives has been previously considered for pharmaceutical active compounds [34] but we do not believe that this strategy has been previously reported for agricultural chemicals. The findings of this study enhance the understanding of the physical properties, including self-assembling behaviour, of the active-amphiphilic conjugates, which in turn helps to improve the design of future constructs for desired behavior. Studies to determine the permeability and efficacy of the novel conjugates in plant-based trials are underway and will provide a new class of agrochemical delivery vectors if effective.

### **Associated content**

#### **Supplementary information**

The  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of synthesized agrochemical-lipid conjugates including PIC-Phyt, PIC-GMO, PIC-GME, PIC-EO3-oleate, PIC-EO4-oleate and 2,4D-GMO. The 2-D SAXS contour graphs and corresponding lattice parameter graphs of dispersed agrochemical-lipid conjugates in phytantriol and GMO matrix. The polarized optical images of agrochemical-lipid conjugates in phytantriol and GMO matrix.

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