# Supporting materials

## Reduction of Colitis in Mice by Chemically Programmed Supramolecular Nanoassemblies of Vitamin–Lipid Conjugates

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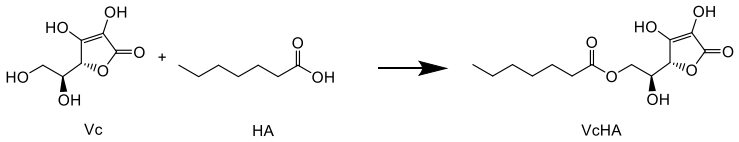
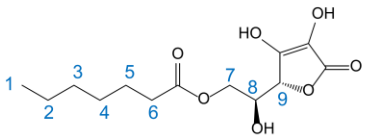
Table S1

## Synthesis of VcHA




$V_C$ (1.7 g, 10.0 mmol) and HA (2.6 g, 20.0 mmol) was dissolved in 25 mL of anhydrous acetonitrile. Then, lipase (0.3 g) and molecular sieve were added. The mixture was warmed up to 55°C and stirred for 12 hours to allow for full reaction. After the reaction, the solvent is volatilized by rotary evaporation under reduced pressure. Next, silica gel was used as a stationary phase, and a gradient of dichloromethane and methanol as eluents was employed for purification by column chromatography to obtain the pure product (1.8 g, 62.3%). $V_C$HA were characterized via $^1$H NMR and HPLC.

$^1$H NMR (400 MHz, Chloroform-d) δ 4.83 (s, 1H), 4.39 (d, *J* = 10.6 Hz, 1H), 4.27 (s, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 1.62 (p, *J* = 7.4 Hz, 2H), 1.31 –1.25 (m, 6H), 0.93 – 0.82 (m, 3H).



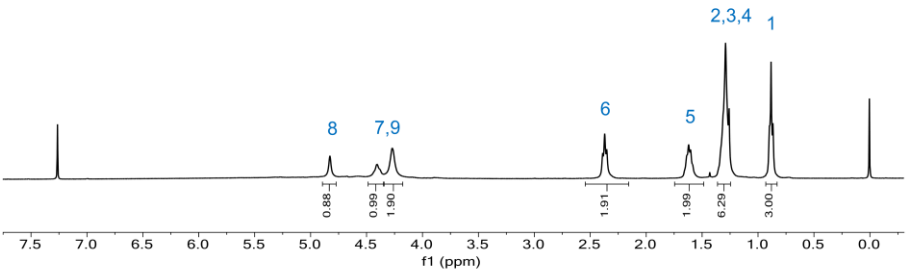
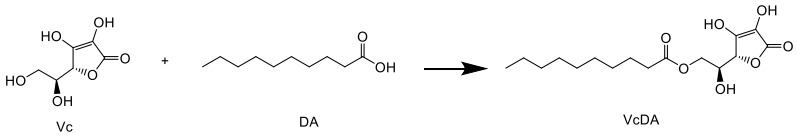
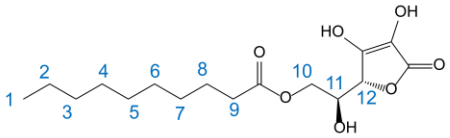




**Figure S1.** $^1$H NMR spectrum of $V_C$HA in chloroform-*d*.

## Synthesis of VcDA




$V_C$DA was synthesized following the same procedure as that employed for the synthesis of $V_C$HA as described above. The reaction utilized 3.5 g (10.0 mmol) of $V_C$, 3.4 g (20.0 mmol) of DA and 0.3 g of lipase as reagents. The resulting product was obtained as a pale yellow oily solid with a yield of 50.0% (1.7 g). The chemical structure of the final product was confirmed through $^1$H NMR spectroscopy.
$^1$H NMR (400 MHz, Chloroform-d) δ 4.83 (s, 1H), 4.40 (s, 1H), 4.26 (s, 2H), 2.36 (t, *J* = 7.7 Hz, 2H), 1.61 (s, 2H), 1.26 (d, *J* = 6.8 Hz, 12H), 0.87 (t, *J* = 6.6 Hz, 3H).



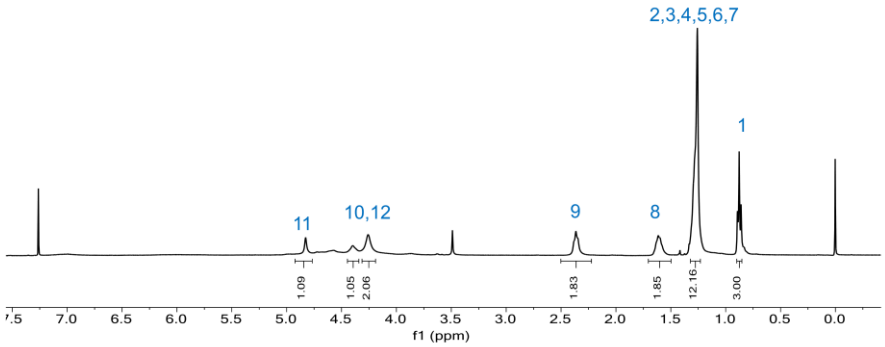
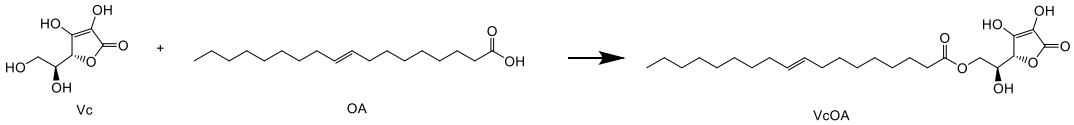



**Figure S2.** $^1$H NMR spectrum of $V_C$DA in chloroform-*d*.

## Synthesis of $V_C$OA




$V_C$OA is synthesized in the same manner as the method described above for synthesizing $V_C$HA. The reaction used 3.5 g of (10.0 mmol) $V_C$, 5.6 g (20.0 mmol) DA and 0.3 g lipase as reagents. The product is a white solid with a yield of 64.9% (2.9 g). The chemical structure of the final product was confirmed by $^1$H NMR spectroscopy.
$^1$H NMR (400 MHz, Chloroform-d) δ 5.43 – 5.24 (m, 2H), 4.81 (s, 1H), 4.40 (q, *J* = 8.9 Hz, 1H), 4.26 (d, *J* = 6.3 Hz, 2H), 2.36 (t, *J* = 7.6 Hz, 2H), 2.01 (q, *J* = 6.4 Hz, 4H), 1.28 (d, *J* = 13.6 Hz, 20H), 0.90 – 0.85 (m, 3H).

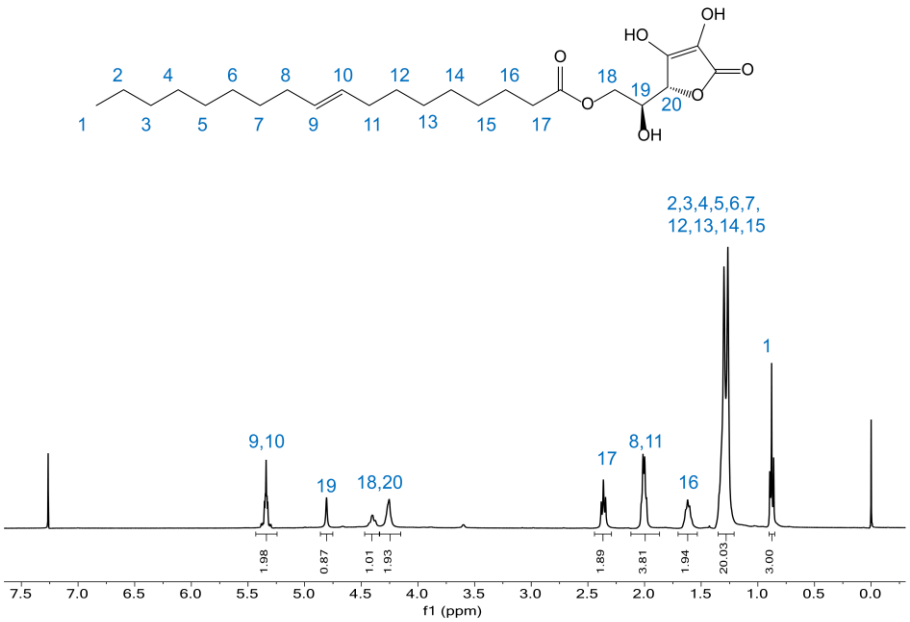
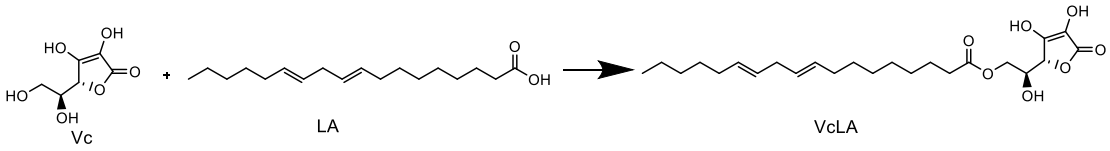



**Figure S3.** $^{1}$H NMR spectrum of $V_C$OA in chloroform-*d*.

## Synthesis of $V_C$LA




The synthesis of $V_C$LA is the same as the $V_C$HA described above. The reaction was added with 3.5 g (10.0 mmol) $V_C$, 5.6 g (20.0 mmol) LA and 0.3 g lipase as reagents. The product was a yellowish oily solid with a yield of 71.1% (3.1 g). The chemical structure of the final product is confirmed by $^{1}$H NMR spectroscopy.

$^{1}$H NMR (400 MHz, Chloroform-d) δ 5.49 – 5.23 (m, 4H), 4.80 (s, 1H), 4.51 – 4.37 (m, 1H), 4.28 (d, *J* = 7.4 Hz, 2H), 2.77 (t, *J* = 6.4 Hz, 2H), 2.37 (t, *J* = 7.6 Hz, 2H), 2.05 (q, *J* = 6.8 Hz, 4H), 1.36 – 1.25 (m, 14H), 0.89 (t, *J* = 6.8 Hz, 3H).

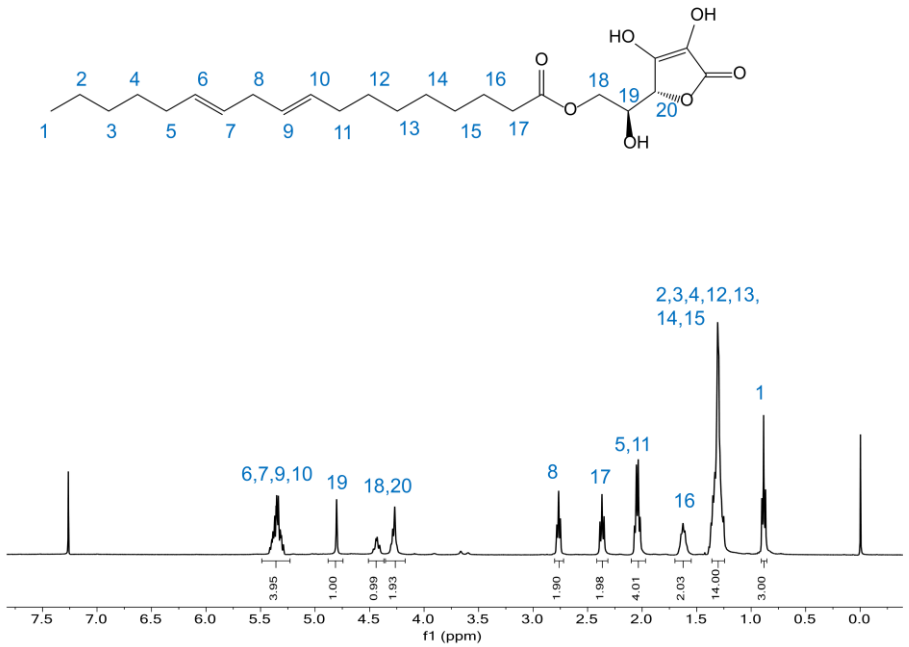
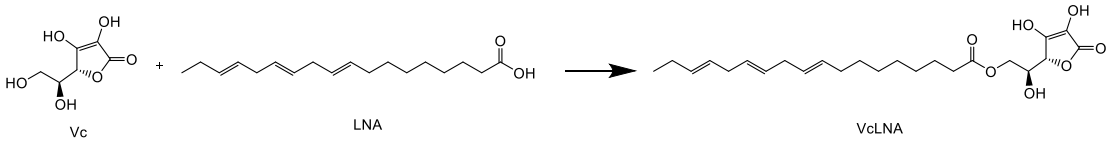



**Figure S4.** $^{1}$H NMR spectrum of V$_C$LA in chloroform-*d*.

## Synthesis of V$_C$LNA




The reaction utilized 3.5 g (10.0 mmol) of V$_C$, 5.6 g (20.0 mmol) of LNA and 0.3 g of lipase as reagents. The resulting product was obtained as a pale yellow oily solid with a yield of 67.5% (3.0 g). The chemical structure of the final product was confirmed through $^{1}$H NMR spectroscopy.

$^{1}$H NMR (400 MHz, Chloroform-d) δ 5.44 – 5.25 (m, 6H), 4.80 (s, 1H), 4.42 – 4.33 (m, 1H), 4.24 (d, *J* = 5.8 Hz, 2H), 2.80 (t, *J* = 6.4 Hz, 4H), 2.36 (t, *J* = 7.6 Hz, 2H), 2.06 (p, *J* = 7.2 Hz, 4H), 1.29 (d, *J* = 6.8 Hz, 8H), 0.97 (t, *J* = 7.6 Hz, 3H).

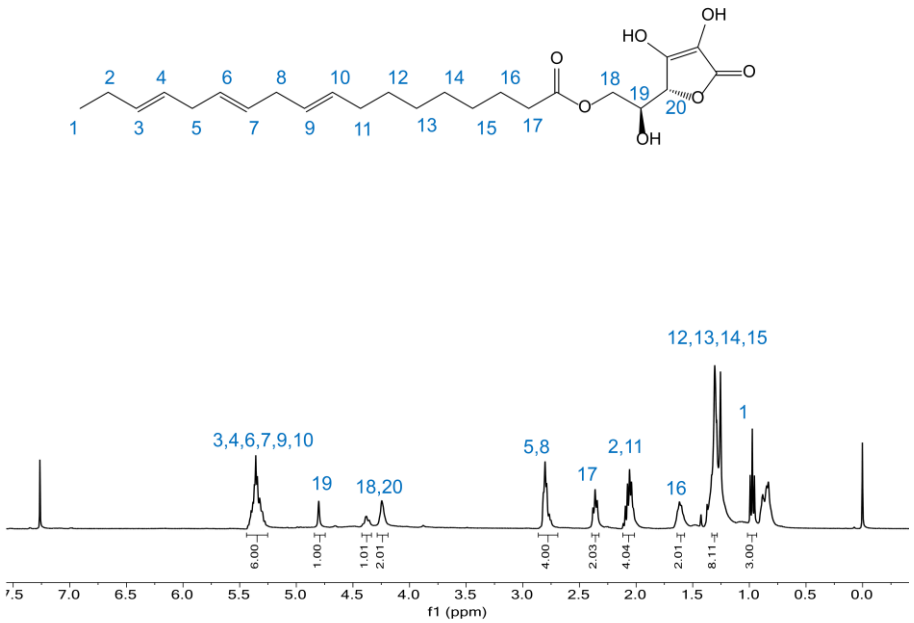
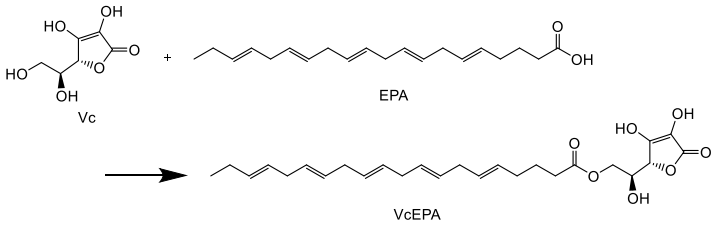



**Figure S5.** $^{1}$H NMR spectrum of V$_{C}$LNA in chloroform-*d*.

## Synthesis of V$_{C}$EPA




The reaction used 3.5 g of (10.0 mmol) V$_{C}$, 6.0 g (20.0 mmol) EPA and 0.3 g lipase as reagents. The product is a white solid with a yield of 65.3% (3.0 g). The chemical structure of the final product was confirmed by $^{1}$H NMR spectroscopy.

$^{1}$H NMR (400 MHz, Chloroform-d) δ 5.38 (qq, J = 12.8, 6.8 Hz, 10H), 4.81 (s, 1H), 4.41 (q, *J* = 8.9 Hz, 1H), 4.33 – 4.19 (m, 2H), 2.91 – 2.71 (m, 8H), 2.38 (t, *J* = 7.8 Hz, 2H), 2.09 (dp, *J* = 14.8, 7.2 Hz, 4H), 1.70 (q, *J* = 7.6 Hz, 2H), 0.97 (t, *J* = 7.6 Hz, 3H).

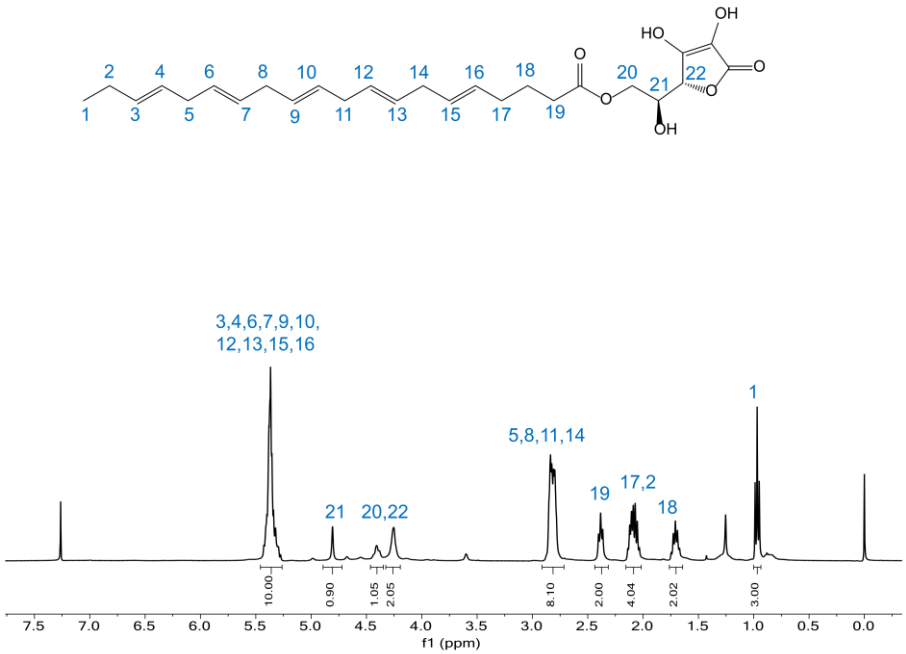
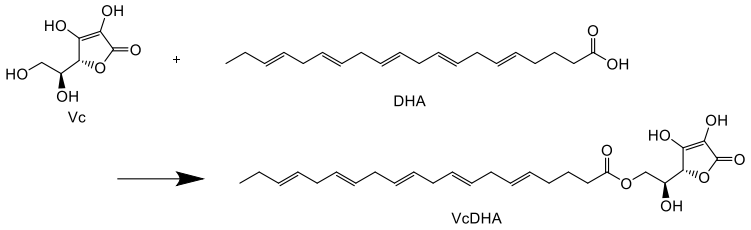



**Figure S6.** $^{1}$H NMR spectrum of $V_C$EPA in chloroform-*d*.

## Synthesis of $V_C$DHA




The reaction was added with 3.5 g (10.0 mmol) $V_C$, 6.0 g (20.0 mmol) LA and 0.3 g lipase as reagents. The product was a yellowish oily solid with a yield of 73.4% (3.4 g). The chemical structure of the final product is confirmed by $^{1}$H NMR spectroscopy. $^{1}$H NMR (400 MHz, Chloroform-d) δ 5.54 – 5.22 (m, 12H), 4.81 (s, 1H), 4.43 (q, *J* = 9.0 Hz, 1H), 4.26 (s, 2H), 2.83 (p, *J* = 6.6, 5.8 Hz, 10H), 2.42 (dp, *J* = 17.6, 6.4, 5.1 Hz, 4H), 2.07 (p, *J* = 7.4 Hz, 2H), 0.97 (t, *J* = 7.4 Hz, 3H).

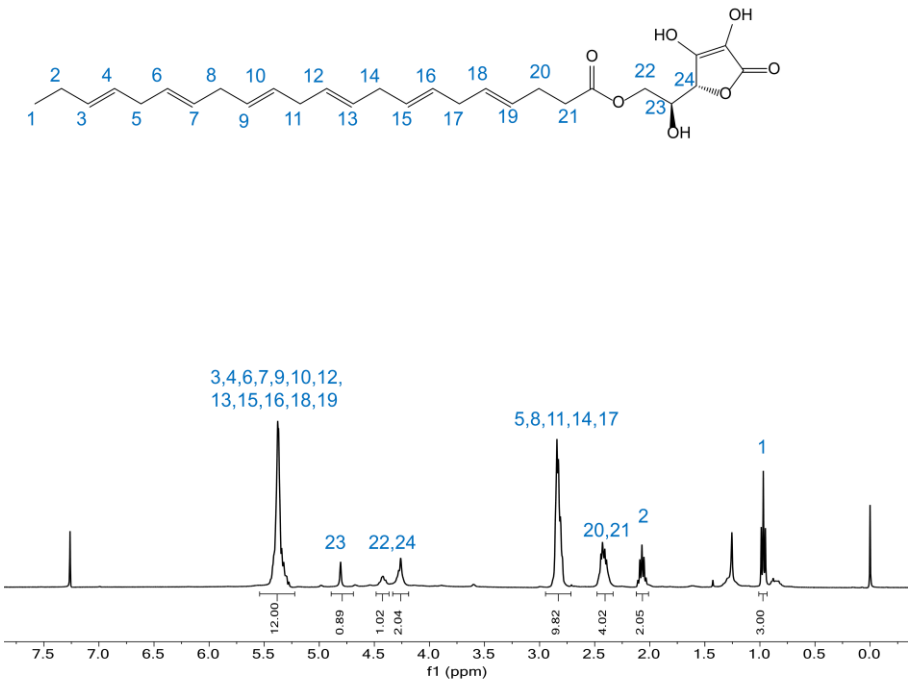
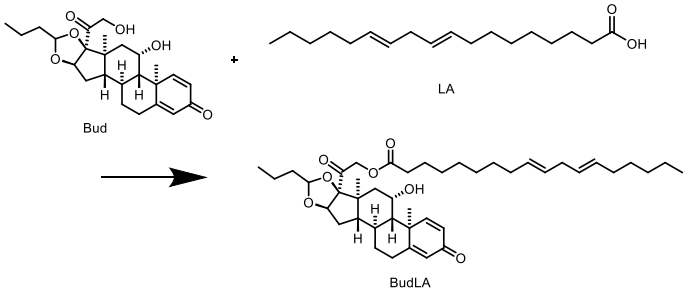



**Figure S7.** $^{1}$H NMR spectrum of $V_C$DHA in chloroform-*d*.

## Synthesis of BudLA

For the preparation of budesonide conjugates, budesonide (4.3 g, 10.0 mmol) and linoleic acids (2.8 g, 10.0 mmol) were mixed in 10 mL DCM. Then 4-Dimethylaminopyridine (1.3 g, 11.0 mmol) and N, N'-diisopropylcarbodiimide (1.4 g, 11.0 mmol) were added into the above solution. The mixture was warmed up to 45°C and stirred for 10 h, and the subsequent purification methods are the same as $V_C$ lipid derivatives. The chemical structure of the final product was confirmed with $^{1}$H NMR and HPLC in our previous study[1].



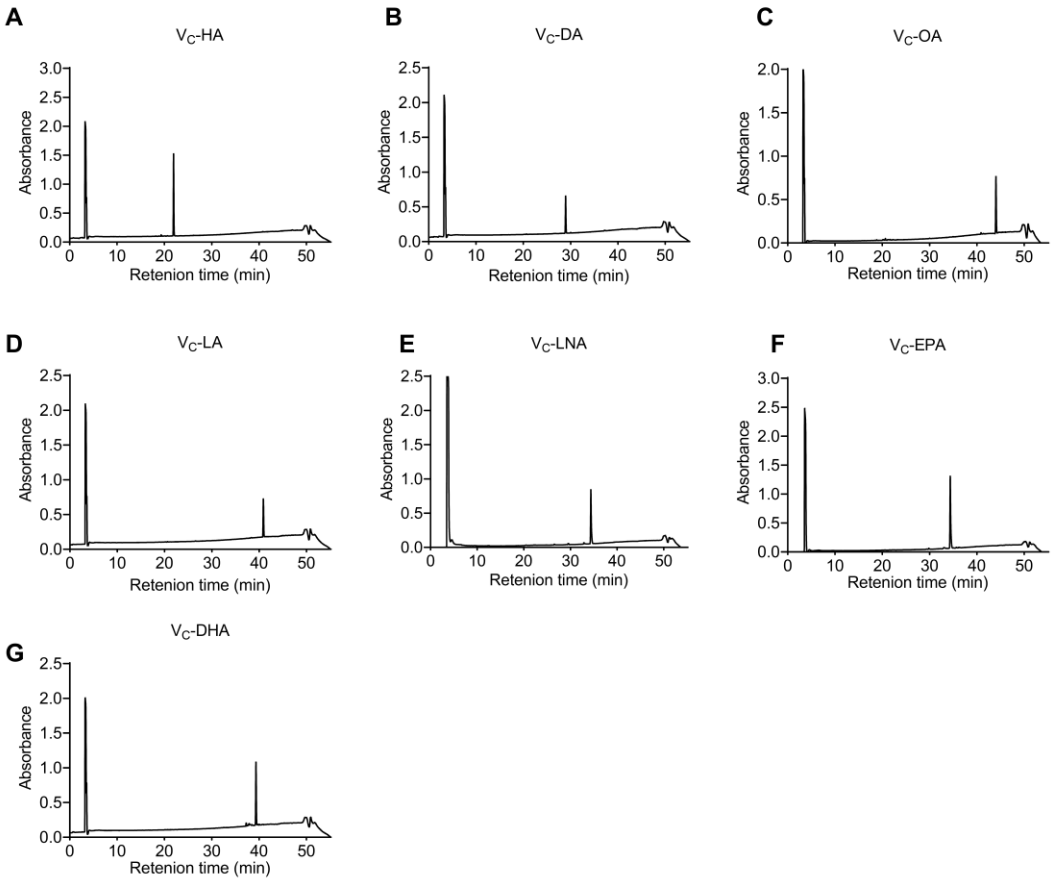



**Figure S8.** HPLC analysis of $V_C$ lipid derivatives**.**

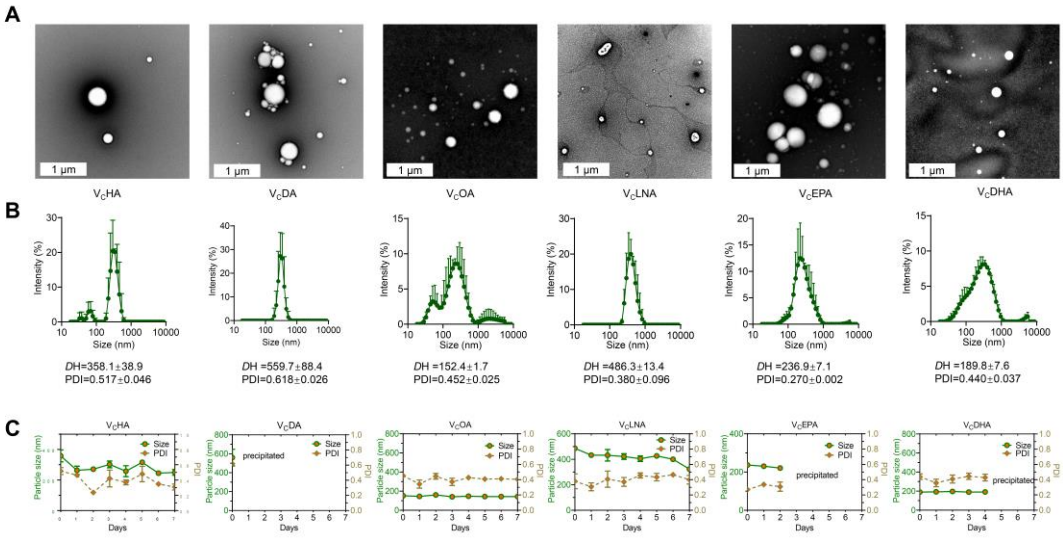



**Figure S9.** Transmission electron microscope images (A), size distribution (B) and stability (C) of $V_C$HA, $V_C$DA, $V_C$OA, $V_C$LNA, $V_C$EPA and $V_C$DHA.

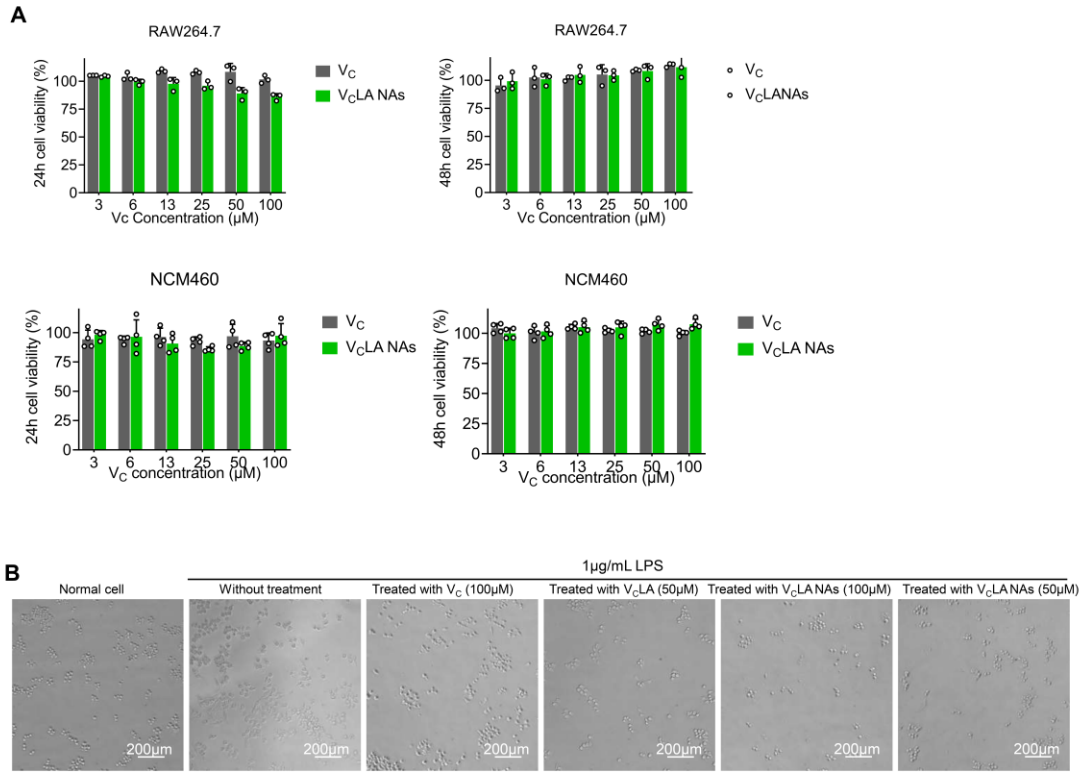



**Figure S10.** (A) 24 h and 48 h cell viability of RAW 264.7 or NCM460 incubated increasing concentration of free $V_C$ (or $V_C$LA NAs) as measured by CCK-8 assay. (B) Representative images of RAW 264.7 cultivated with different agents (PBS, LPS, LPS+50/100μM $V_C$, LPS+50/100μM $V_C$LA Nas) for 24h.

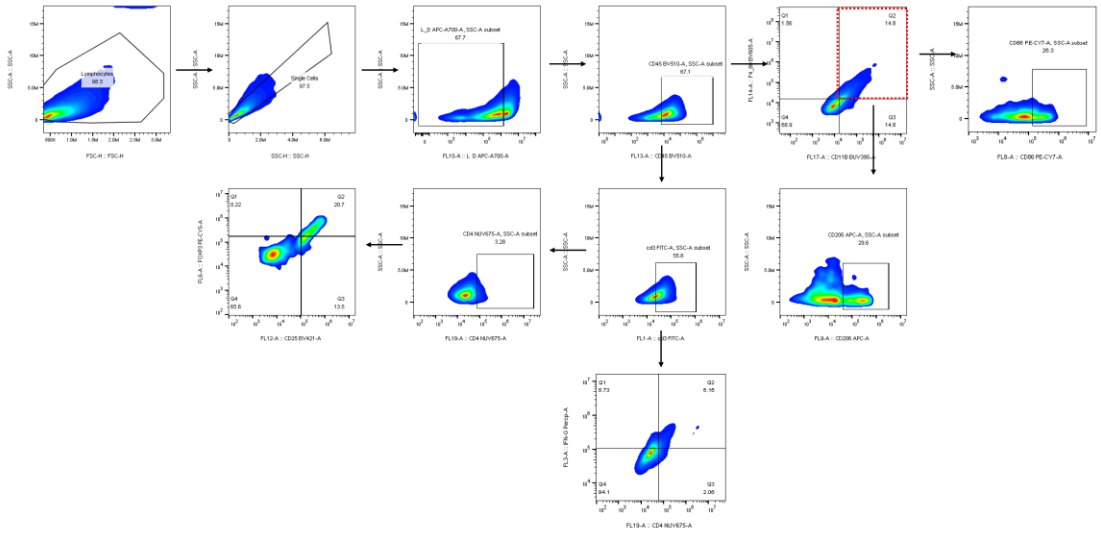


**Figure S11.** Flow cytometry gating strategy for analysis of $CD11b^+F4/80^+$cells, $F4/80^+CD86^+$cells, $F4/80^+CD206^+$cells, $CD25^+FoxP3^+$ cells and $CD4^+$IFN-$\gamma^+$ in colon of mice.

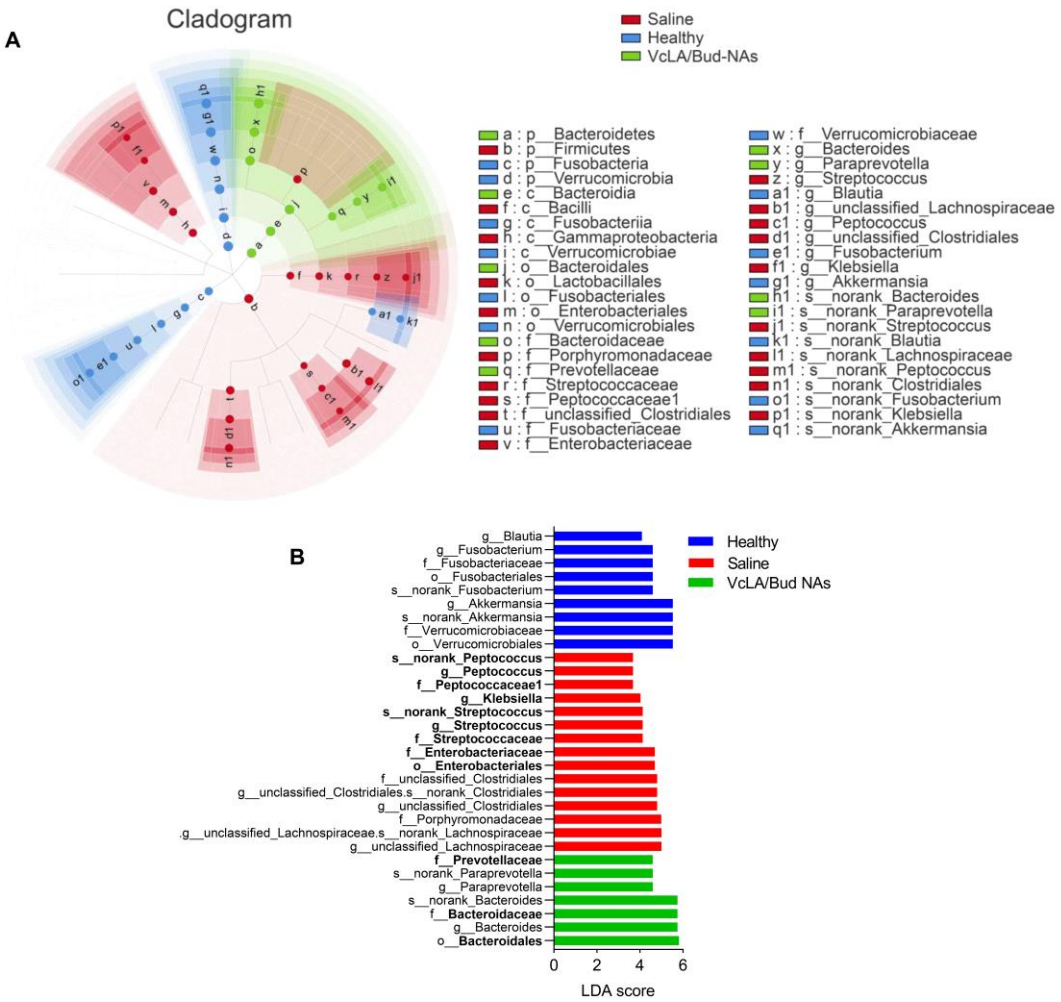



**Figure S12.** (A) LefSe analysis cladogram representing the significantly different taxas among three groups at the end of the treatment (LDA > 2, $p < 0.05$). (B) LDA score of microbiota.

**Table S1**. Scoring system for DAI.

| Score | Weight loss | Stool consistency | Fecal occult blood |
|---|---|---|---|
| 0 | no loss | normol | no blood |
| 1 | 1-5% | | |
| 2 | 5-10% | loose stool | presence of blood |
| 3 | 10-15% | | |
| 4 | >15% | watery diarrhea | visible bleeding |

## Reference

1. Xian, S., et al., Oral liposomal delivery of an activatable budesonide prodrug reduces colitis in experimental mice. Drug Deliv, 2023. 30(1): p. 2183821.