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ORIGINAL PAPER

Clickable Lipids: Azido and Alkynyl Fatty Acids and Triacylglycerols

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Abstract Hydroxy fatty acids (FAs), which were isolated from glycolipids that can be prepared fermentatively from fats and oils, have been synthetically modified to contain azide and alkyne functional groups. These particular functional groups were chosen because they can participate in a copper-catalyzed reaction that combines them to form a 1,4-triazole, known as a "click" reaction, which has been widely used in a variety of fields but remains underutilized in FA chemistry. Depending on the starting hydroxy FA, these groups can be close to the carboxy unit (using 3-hydroxydecanoate) and hence the polar glycerol group, or distant from it (using 17-hydroxyoctadecanoate). These structural alternatives will impart different properties to the triacylglycerols that are subsequently prepared from the modified FA. Finally, the click reaction was used to conjugate triacylglycerols to each other and to other molecules such as a glycolipid or a protected amine.

Keywords Triacylglycerols · Hydroxy fatty acids · Functionalized lipids · Click reaction

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Introduction

Triacylglycerols (TAGs), in addition to their fundamental importance to cellular biology, have been receiving increased attention as building blocks for a variety of self-assembled structures. Materials such as tailored liposomes [1], lipid nanotubes [2], or archaeosomes [3] have interesting properties and rely upon fatty acids (FAs) as principal components. Lipids can also be used for templating inorganic materials [4] or, when supported on a solid layer, in the design of biosensors [5]. Synthetic or structural advances in FA may therefore have significant impact on the range of advanced materials that can be constructed. Furthermore, modified or labeled TAGs can be used to address basic questions about the structure and dynamics of lipid membranes [6].

Our previous work has investigated synthetic modification of, and uses for, 17-hydroxystearic acid (Scheme 1), which can be obtained in good yield after hydrolysis of sophorolipids produced by fermentation of agricultural byproducts [7]. We reasoned that the particular structural features of this molecule—namely, the positioning of its reactive sites at opposite ends of the 18-carbon chain—would confer novel properties when incorporated into TAGs. A contrasting pattern of reactive sites was provided by the 3-hydroxy FAs obtained from hydrolysis of another type of glycolipid, namely rhamnolipids, also obtained fermentatively [8]. In particular, our objective was to use hydroxy FAs **1a** and **2a** as means to expand the use of "click" chemistry with TAG-based materials. The copper-catalyzed coupling of azides and alkynes, commonly known as a "click" reaction, has proven to be exceptionally versatile in a wide range of situations [9]. While it has found innovative use in preparing membrane-associated materials such as lipoproteins or oligonucleotides,

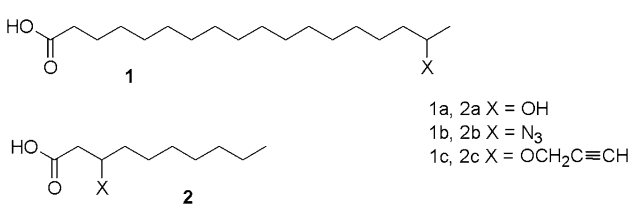
particularly when the polar headgroup is modified to contain an azide or alkyne [10–13], there are still only a few examples where derivatized FAs containing these functionalities, especially when they are distant from the head group, are used to prepare TAGs. One such example is Smith's work on membrane-spanning ion-conductive lipids [14], and another is the hydroxy azides derived from epoxidized soy oil [15]. We wish to report synthetic modifications of **1a** and **2a** into azide and alkyne derivatives and their incorporation into TAGs. These novel TAGs, once subjected to click conditions, can serve as carriers for a large range of classes of "biomolecular cargo" [14] or can be covalently attached to a surface, opening up new lines of study in the applications listed above.

Experimental

General

Methyl 17-hydroxystearic acid was obtained from sophorolipids as described previously (briefly, heating with H_2SO_4 in methanol) [7]; methyl 3-hydroxydecanoate was obtained analogously from rhamnolipids [8]. Chemicals and reagents were obtained commercially from Sigma–Aldrich (St Louis, MO, USA) and Lancaster Synthesis (Alfa Aesar, Ward Hill, MA, USA). All solvents and reagents were used as received. Silica gel (grade 60 Å, mesh 230–400, particle size 40–63 μm), used for all column chromatography, was obtained from Fisher Scientific (Fairlawn, NJ, USA). Solvents were removed on a rotary evaporator. NMR spectra were recorded on either a Varian Associates (Walnut Creek, CA, USA) Gemini 200 MHz or Inova 400 MHz instrument and are referenced to $Si(CH_3)_4$. LC–MS data were recorded on a Waters–Micromass (Milford, MA, USA) ZMD instrument with APCI, using either:

1. an elution gradient of 40:60 water–acetonitrile to 100% acetonitrile over 30 min (or minor variations on those conditions) on a 2.1 × 150 mm Waters Symmetry C_{18} 3.5 μm column; or
2. direct injection without an LC column.



Scheme 1 Structures of the starting materials

Matrix-assisted laser desorption/ionization mass spectra with automated tandem time of flight fragmentation of selected ions (MALDI–TOF–TOF) were acquired with an ABI 4700 Proteomics Analyzer mass spectrometer (Applied Biosystems, Framingham, MA, USA) in the positive-reflection mode. Masses were determined as sodiated adducts of the products, $[M + Na]^+$, using 2,5-dihydroxybenzoic acid as matrix at a concentration of 10 mg/mL in acetonitrile–water (1:1) containing 0.1% TFA. Approximately 0.7 μL matrix was spotted on the MALDI plate and allowed to dry. The sample (0.5–1 μL) dissolved in chloroform or acetone at a concentration of 1–2 mg/mL was spotted on the top of the matrix crystals. Averages of 1,000–2,000 spectra were acquired for optimal signal-to-noise ratio.

*Synthesis of 17-Azido Stearic Acid (**1b**)*

To a solution of methyl 17-hydroxystearate (1.0 g, 3.2 mmol), diisopropylazodicarboxylate (0.84 g, 4.1 mmol), and triphenylphosphine (1.1 g, 4.1 mmol) in 25 mL THF, diphenylphosphorylazide (1.16 g, 4.21 mmol) in 3 mL THF was added dropwise [16]. The reaction was stirred at RT overnight, then solvent was removed. The crude material was purified by column chromatography with 4:1 hexane–diethyl ether to provide the methyl ester of **1b** (R_f 0.8, 0.74 g, 68%). The ester was converted to the free acid by dissolving it in methanol and THF and adding an aqueous solution of LiOH (approximately two equivalents). The reaction was heated at 50 °C for 2 h then stirred at RT overnight. Solvent was removed, then the aqueous layer was acidified with citric acid and extracted with ethyl acetate to afford **1b** (0.68 g, 96%) which was used without further purification.

*Synthesis of 3-Azidodecanoic Acid (**2b**) [17, 18]*

A solution of methyl 3-hydroxydecanoate (1.2 g, 5.9 mmol) and *p*-toluene sulfonic acid (25 mg, 0.15 mmol) was heated to reflux in 30 mL 2-methyl-1-propanol (isobutanol) for 24 h. After removal of solvent, the material was purified by column chromatography in 4:1 hexane–ethyl acetate (R_f product 0.5, starting material 0.4). The isobutyl 3-hydroxydecanoate **3** thus obtained (1.37 g, 5.61 mmol, 94%) was dissolved in 10 mL dichloromethane and pyridine was added (1 mL) followed by methanesulfonyl chloride (0.96 g, 8.4 mmol). Reaction proceeded at RT overnight, after which the solvent was removed. The isobutyl 3-mesyldecanoate **4** was heated in 20 mL 2 M HCl at 55 °C for 48 h. After the solution cooled to RT, it was extracted with dichloromethane (3 × 25 mL). The organic solution was dried over $MgSO_4$ and solvent was removed. TLC of this crude material showed that some

starting material (isobutyl ester) remained, but the crude product was used as is. The 3-mesyldecanoic acid **5** was dissolved in 10 mL DMSO that had been dried over activated molecular sieves, sodium bicarbonate (0.5 g, 6 mmol) was added, and the mixture was heated to 50 °C. After 3 h, sodium azide was added, and heating was continued for 18 h. The mixture was cooled to RT and extracted with 200 mL diethyl ether and 100 mL water. The ether layer was condensed and the resulting **2b** (880 mg, approximately 85% pure as estimated by ^{1}H NMR spectroscopy, 63% from **3**) was used as described below for preparation of TAGs without further purification (Fig. 1).

*Synthesis of Propargyl Ethers **1c** and **2c***

A representative procedure is given for **1c**: propargyl alcohol (0.96 g, 1.0 mL, 17 mmol) and trichloroacetonitrile (2.88 g, 2.0 mL, 20.0 mmol) were dissolved in 25 mL CH_2Cl_2 and one drop of 1,8-diazabicycloundecane (DBU) was added. The mixture was stirred at RT for 1 h, then passed through a short plug of silica gel which was washed with ethyl acetate to remove the DBU. The organic solvents were removed. The propargyl trichloroacetimidate thus obtained was dissolved in 5 mL 2:1 cyclohexane–dichloromethane, and the methyl ester of **1a** was added (1.0 g, 3.2 mmol). Trifluoromethanesulfonic acid (50 μL) was added and the solution was stirred overnight [19]. Solvent was removed and the crude product was purified by column chromatography with 4:1 hexane–ethyl ether (product had $R_f = 0.4$, 830 mg, 74%). This methyl ester of **1c** was converted to the free acid as for **1b** above, with LiOH.

General Route for the Synthesis of Acylglycerol Species

To a solution of 1-monostearin (1.0 g, 2.8 mmol), 10-undecylenic acid (0.46 g, 2.5 mmol), and *N*,*N*-diisopropylethylamine (0.87 mL, 5.0 mmol) in 25 mL THF was added benzoyl chloride (0.29 mL, 2.5 mmol), followed 5 min later by 4-dimethylaminopyridine (DMAP, 60 mg) [20]. The reaction was stirred at RT overnight, then solvent was removed and the crude product was purified by column chromatography with 3:1 hexane–ethyl acetate. A small amount of triacylglycerol (stearic/di-undecylenic) eluted early, followed by two isomers of DAG, which could be clearly distinguished by NMR. The 1,3-isomer eluted first ($R_f = 0.4$, 811 mg, 62%) followed by the 1,2 isomer ($R_f = 0.3$, 322 mg, 25%). The 1,3-DAG was then used in an analogous procedure with **1b**, **1c**, **2b**, or **2c** to prepare TAGs, with yields ranging from 74 to 89%.

Synthesis of 1,4-Triazole Adducts (The "Click" Reaction)

Equimolar amounts of an azido TAG and an alkynyl TAG were combined in 10 mL 2:1 H_2O–t-BuOH. If the TAGs seemed to be insoluble, THF was added until they dissolved. The solution was stirred vigorously, and an aqueous solution of $CuSO_4{\cdot}5H_2O$ (10 mol%) was added, followed by an aqueous solution of sodium ascorbate (20 mol%) [21]. TLC at 1 h generally indicated the reaction was complete, but reaction was usually continued overnight. Organic solvents were removed, and the aqueous residue was extracted with ethyl acetate, which was again removed under vacuum, and the residue chromatographed. Yields were ≥94%.

Spectroscopic Data

NMR spectra were recorded at RT in $CDCl_3$

6: ^{1}H: 0.79–0.94 (m, 6H, CH_3), 1.11–1.38 (m, 48H), 1.45 (s, 9H, Boc), 1.51–1.70 (m, 4H), 1.80–1.95 (m, 2H), 1.95–2.14 (m, 2H, C*H*$_2$–CH=), 2.21-2.38 (m, 4H, C(O)–CH_2), 2.69–2.87 (m, 2H, CH_2 next to C(O)–piperazine), 2.97–3.18 (m, 4H, C(2)*H*$_2$–C(3)H–N and CH_2 next to triazole), 3.32–3.53 (m, 4H, piperazine), 3.53–3.66 (m, 4H, piperazine), 3.99–4.37 (m, 4H, glycerol CH_2), 4.71–4.85 (m, 1H, C(3)H-triazole), 4.85–5.06 (m, 2H, CH=C*H*$_2$), 5.12–5.23 (m, 1H, glycerol CH), 5.67–5.83 (m, 1H, C*H*=CH_2), 7.40 (s, 1H, triazole). ^{13}C: 14.1, 14.6, 21.1,

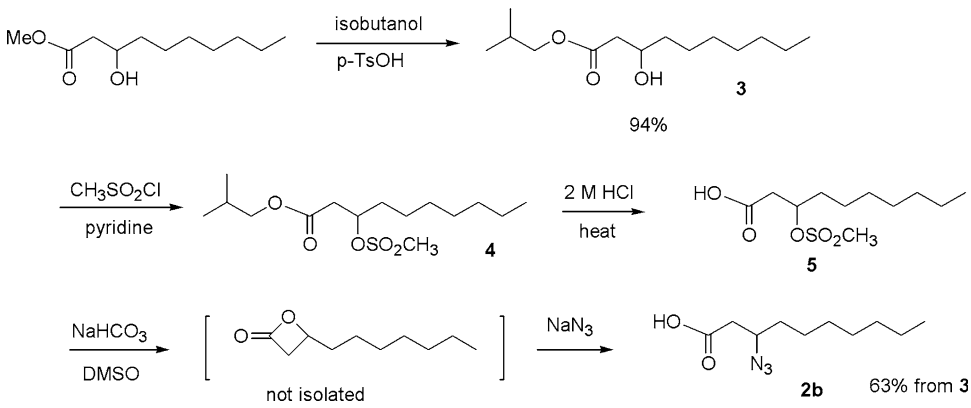


Fig. 1 Synthetic route used to convert 3-hydroxydecanoic acid to 3-azidodecanoic acid

22.6, 22.7, 24.9, 25.9, 28.4, 29.0–29.8, 31.7, 32.0, 32.3, 32.5, 33.8, 34.0, 35.2, 39.9, 41.6, 43.6 (piperazine), 45.3, 57.9 (CH-triazole), 61.8 (glycerol CH_2's), 69.9 (glycerol CH), 80.4 (Boc C–O), 114.2 (CH=CH_2), 121.5 (triazole CH),139.2 (CH=CH_2), 146.2 (triazole C), 154.6 (Boc C=O); 169.4, 169.6, 170.6, 173.3 (carbonyls). APCI: calculated for $C_{56}H_{99}N_5O_9H^+$ 986.74, found 986.62.

7: ^{1}H: 0.82–0.98 (t, 7.0 Hz, 12H, CH_3), 1.11–1.48 (m, 94H), 1.51–1.73 (m, 12H), 1.81–1.99 (m, 2H), 1.99–2.13 (m, 4H, CH_2–CH=), 2.24–2.38 (m, 8H, C(O)–CH_2), 2.43-2.72 (m, 2H, C(2)H_2–C(3)H–O), 3.01 (m, 2H, C(2)H_2–C(3)H-triazole), 3.85–3.94 (m, 1H, C(3)H–O), 4.04–4.39 (m, 8H, glycerol CH_2), 4.67 (s, 2H, O–CH_2), 4.77–4.90 (m, 1H, C(3)H–N), 4.90–5.06 (m, 4H, CH=CH_2), 5.15–5.36 (m, 2H, glycerol CH), 5.72–5.94 (m, 2H, CH=CH_2), 7.61 (s, 1H, triazole). ^{13}C: 14.1, 22.6, 22.7, 24.8, 25.2, 25.8, 28.9–29.7, 31.7, 31.9, 33.8, 33.9, 34.0, 35.1, 39.5, 39.9, 57.9 (C(3)H-triazole); 61.8, 61.9, 62.0, and 63.1 (glycerol CH_2's and O–CH_2-triazole); 69.2 and 69.9 (glycerol CH's), 76.0 (C(3)H–O), 114.2 (CH=CH_2), 122.3 (triazole CH), 139.1 (CH=CH_2), 145.0 (C-triazole); 169.4, 170.7, 173.2, 173.3 (carbonyls). MALDI: calculated for $C_{87}H_{157}N_3O_{13}H^+$ 1,453.18, found 1,453.17.

8: ^{1}H: 0.88 (t, 6.7 Hz, 6H, CH_3), 1.12–1.47 (m, 118H), 1.19 (d, 6.3 Hz, 3H), 1.50–1.72 (m, 12H), 1.54 (d, 6.9 Hz, 3H), 1.77–1.93 (m, 2H), 1.93–2.13 (m, 8H, CH_2–CH=), 2.32 (t, 7.6 Hz, 12H, C(O)–CH_2), 3.48–3.61 (m, 1H, CH–O), 4.09–4.37 (m, 8H, glycerol CH_2), 4.56–4.75 (m, 3H, O–CH_2 and CH–N), 4.89–5.07 (m, 4H, CH=CH_2), 5.22–5.39 (m, 4H, glycerol CH and CH=CH), 5.71–5.93 (m, 2H, CH=CH_2), 7.51 (s, 1H, triazole). ^{13}C: 14.2, 19.7, 21.5, 22.8, 25.0, 25.7, 26.0, 27.2, 27.3, 29.0–29.8, 32.0, 33.9, 34.1, 34.3, 36.6, 37.3, 57.4 (C(17)H-triazole), 62.2 and 62.4 (glycerol CH_2's), 69.0 (glycerol CH), 75.6 (C(17)H–O), 114.2 (CH=CH_2), 119.9 (triazole CH), 129.8 and 129.9 (CH=CH), 139.2 (CH=CH_2), 146.0 (triazole C), 172.9 and 173.3 (carbonyls). MALDI: calculated for $C_{103}H_{187}N_3O_{13}H^+$ 1,675.41, found 1,675.44.

9: ^{1}H: (assignment letters in bold caps are keyed to Fig. 2 and Scheme 2): 0.90 **A** (t, 6.8 Hz, 6H, CH_3), 1.12–1.41 (m, 159H), 1.46 **B** (s, 18H, Boc), 1.51–1.72 (m, 12H), 1.56 (d, 6.9 Hz, 3H), 1.75–1.93 (m, 2H), 1.94–2.09 **C** (m, 4H, CH_2–CH=), 2.32 **D** (t, 7.6 Hz, 12H, C(O)–CH_2), 2.51 **E** (t, 6.0 Hz, 4H, β-Ala C(O)–CH_2), 3.40 **F** (q, 6.3 Hz, 4H, β-Ala CH_2–N), 3.57 **G** (q, 6.0 Hz, 1H, CH–O), 4.09–4.37 **H** (m, 8H, glycerol CH_2), 4.56–4.75 **I** and **J** (m, 3H, CH_2–O and CH–N), 4.85–5.10 **K** and **L** (m, 4H, C(17)H–O esterified and NH), 5.21–5.42 **M** and **N** (m, 4H, glycerol CH and CH=CH), 7.51 **O** (s, 1H, triazole). ^{13}C: 14.1, 19.5, 19.9, 21.3, 22.6, 24.8, 25.4, 25.9, 27.1, 28.3, 29.1–29.6, 31.9, 34.0, 34.8, 35.9, 36.2, 36.5, 37.2, 57.3 (C(17)H-triazole), 62.0 and 62.2 (glycerol CH_2's and OCH_2-triazole), 68.8 (glycerol CH), 71.4 (C(17)H–O–C=O), 75.4 (C(17)H–OCH_2-triazole), 79.2 (Boc C–O), 119.7 (triazole CH), 129.7 (C=C), 145.8 (triazole C), 155.8 (Boc C=O); 172.1, 172.8, and 173.2 (carbonyls). MALDI: calculated for $C_{133}H_{245}N_5O_{21}Na^+$ 2,271.82, found 2,271.81.

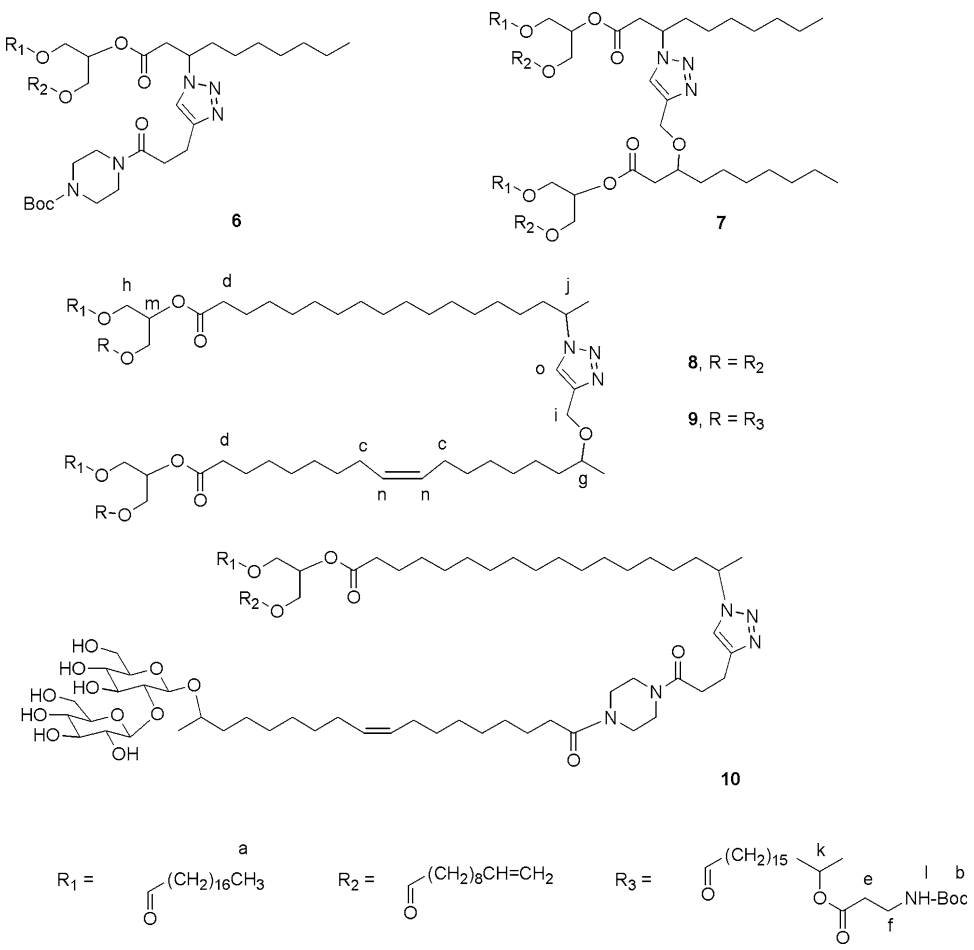
10: ^{1}H: 0.83–0.97 (m, 3H, CH_3), 1.06–1.29 (br s, 90H), 1.30–1.75 (m, 11H), 1.78–1.93 (m, 2H), 1.93–2.15 (m, 6H CH_2–CH=), 2.31 (t, 6.4 Hz, 8H, C(O)–CH_2), 2.81 (t, 6.4 Hz, 2H, CH_2–piperazine), 3.05 (t, 7.6 Hz, 2H, CH_2-triazole), 3.38–4.02 (m, 20H, piperazine and sophorose H except for α-CH), 4.08–4.38 (m, 4H, glycerol CH_2), 4.41–4.57 (m, 1H, C(17)H–O), 4.57–4.73 (m, 1H, CH–N), 4.88-5.09 (m, 2H, CH=CH_2), 5.20–5.42 (m, 5H, glycerol CH, sophorose α-CH's, and CH=CH), 5.71–5.95 (m, 1H, CH=CH_2), 7.36 (s, 1H, triazole). ^{13}C: 15.6, 22.8, 23.1, 24.0, 26.2, 26.5, 27.3, 28.5, 30.4, 30.7, 31.0, 33.2, 33.7, 34.1, 35.1, 38.0, 38.3, 46.4, 46.7, 58.0 (C(17)H–N); 62.6, 62.8, and 63.5 (glycerol CH_2's and sophorolipid CH_2OH and C(17)O); 70.5, 71.7, 76.9, 77.8, 78.0, 78.3, and 78.8 (sophorolipid CH and glycerol CH); 102.9 and 105.9 (sophorolipid α-C's), 116.0 (CH=CH_2), 121.7 (triazole CH), 131.3 (C=C), 140.3 (CH=CH2), 147.6 (triazole C); 171.7, 172.5, 173.5, and 173.9 (carbonyls). MALDI: calculated for $C_{89}H_{159}N_5O_{19}Na^+$ 1,625.15, found 1,625.19.

Results and Discussion

To prepare the azido FAs **1b** and **2b**, we intended to use the Mitsunobu reaction with diphenylphosphoryl azide (DPPA) [16]. This procedure worked well to give **1b**, but the well-known sensitivity of 3-substituted carboxylic acids to elimination prevented its use for **2b** (that is, methyl 2-decenoate was obtained). Instead, we found a more circuitous route adapted from preparation of 3-azido butyrate (Fig. 1) that involves nucleophilic opening of the transient β-lactone [17, 18]. The alkynyl FAs **1c** and **2c** were readily prepared, although an initial attempt relying on base-mediated reaction with propynyl bromide gave only moderate amounts of product plus many side products. Instead, an acid-catalyzed route using the trichloroacetimidate of propynyl alcohol [19] worked well at either the 3 or 17-position. In general, FA-derived azides and alkynes should be readily available through the DPPA and trichloroacetimidate procedures unless a structural feature such as predisposition to elimination necessitates a longer route; it would be interesting to see if a 4-hydroxy FA worked with DPPA.

Preparing TAGs was straightforward. We relied upon the benzoyl chloride-mediated Yamaguchi reaction, which uses inexpensive and simple reagents to make ester bonds [20]. 1-Mono stearin was our starting monoacylglycerol of choice, and most of the TAGs incorporated ω-undecylenic acid as the second FA, because the terminal alkene

Scheme 2 Structures of products



provided a convenient NMR pattern. A mixture of 1,3 and 1,2 DAGs was obtained in a ratio of approximately 3:1, but these could be readily separated from each other by silica gel chromatography. The azido or alkynyl FA was then incorporated similarly. The only problem we encountered with this route was with **2b**. Because the Yamaguchi coupling reaction relies upon DMAP catalysis, presumably forming the highly activated DMAP-carbonyl adduct, elimination from the 3-position is again a problem, and with **2b** we observed the decenoate-containing TAG containing no azide group. We circumvented this problem by switching to a carbodiimide coupling for this example, with hydroxybenzotriazole present to suppress basicity and a lower (5 mol%) amount of DMAP. Using this method, we observed none of the decenoate-containing TAG that occurred via elimination. Because the 1,3-DAG was the more abundant synthetic intermediate, we used it, giving TAGs with the azido or alkynyl FA incorporated at the 2-position, but if for some reason the derivatized FA were desired at a primary glycerol position, the 1,2-DAG could have been used instead.

The alkyne–azide "click" coupling to form a 1,4-triazole was quite simple, as suggested by many literature reports [9, 21]. The two components were combined in 2:1 H_2O–t-BuOH, and THF was added if necessary to solubilize them. Solutions of the copper and reductant reagents were then added. TLC indicated that the reactions were complete in approximately 1 h. The triazole product eluted from silica gel quite distinctly from any unreacted TAG (if one component was present in excess), so clean-up was trivial. This ease of preparation is one strong reason to prefer the click reaction for tethering groups to TAGs. Another is the mildness of the reaction conditions, so other sensitive functional groups that may be present are unaffected. A third advantage is that the reacting partners, the azide and alkyne, are "silent" to, or unchanged by, many reaction conditions for preparing the remainder of the molecules (hydrogenation would be one of the few exceptions). While other reactions for connecting groups are imaginable—epoxide opening or maleate addition by a nucleophile, or amide or ester formation—the functional groups necessary to perform such couplings may be more sensitive and could interfere with other parts of the molecule, or require more elaborate protection schemes. Introducing an epoxide unit, for example, requires an oxidant that may destroy other groups in the molecule or be

non-selective for a particular alkene, and when the epoxide is present, it would be subject to premature opening by other nucleophiles (e.g. alcohols) used in construction of the TAG. In other cases, it may be synthetically very demanding to introduce, for example, a carboxylic acid intended for ester or amide formation. At any rate, our use of the click reaction is not intended to supplant other tactics for lipid design, but it does offer a robust alternative. We have not investigated the long-term stability of the triazole products, but it is worth noting that a sample of compound **9** had its ^{1}H NMR spectrum unchanged after sitting as a solid at room temperature for two months.

We targeted molecules **6** through **10** (Scheme 2) to demonstrate a variety of structural themes that 3 or 17-substitution of TAGs could provide when allied with click chemistry. The major difference, of course, is attachment of a new unit at either the polar end, for the 3-position, or at the non-polar end, for the 17-position. There has been scant systematic investigation of lipids differing in substitution at these positions [22]. Molecule **6** shows how a peptide-like moiety would fit. This substitution pattern has the benefit, relative to phosphatidylcholine-related molecules, of not "using up" one of the glycerol hydroxy groups to attach the charged group. In other words, a polar and/or charged group (here, the masked piperazine) is off to one side, and there are three FA chains anchoring it to a membrane. The number of anchoring hydrophobic chains has been shown to be important for attachment of lipopeptides to a membrane (two being essential) [13], so these clickable TAGs may offer advantages in this area. New types of surfactants could be developed on the basis of such a structural motif; the click reaction, if used with a dialkyne or diazide, would lend itself to construction of gemini surfactants. Similarly, the dimeric molecule **7** is linked at its polar top, whereas **8** is linked at the bottom. It remains to be seen whether a dimer like **8** would orient itself in a bilayer membrane in a spanning mode or with both glycerol units at the same face. A similar question arises for **9** and **10**. In **9** we replaced the undecylene units with extra 17-hydroxystearate derivatives that were esterified with an amino acid. Figure 2 shows that even with a molecule of this size and complexity, the NMR spectrum is readily interpretable; compared with the alkyne precursor (middle spectrum) the terminal alkyne CH resonance has disappeared, with that proton now appearing as the aromatic triazole resonance. The covalent linkage of two TAGs provided by the triazole may be able to stabilize a situation where the charges of the deprotected β-alanine NH_3^+ groups, if countered by a dianion, could reside at the non-polar interlayer region of a lipid bilayer. By varying the size and type of amino acid attached, it may be possible to engineer sites for molecular recognition of ions or hydrogen-bonding groups within a lipid bilayer. The triazole unit itself is polar and capable of hydrogen bonding [9], a feature which could have novel effects on the membrane permeability of fluorophores attached to it [6]. Finally, **10** suggests what may be a general motif for anchoring carbohydrates to

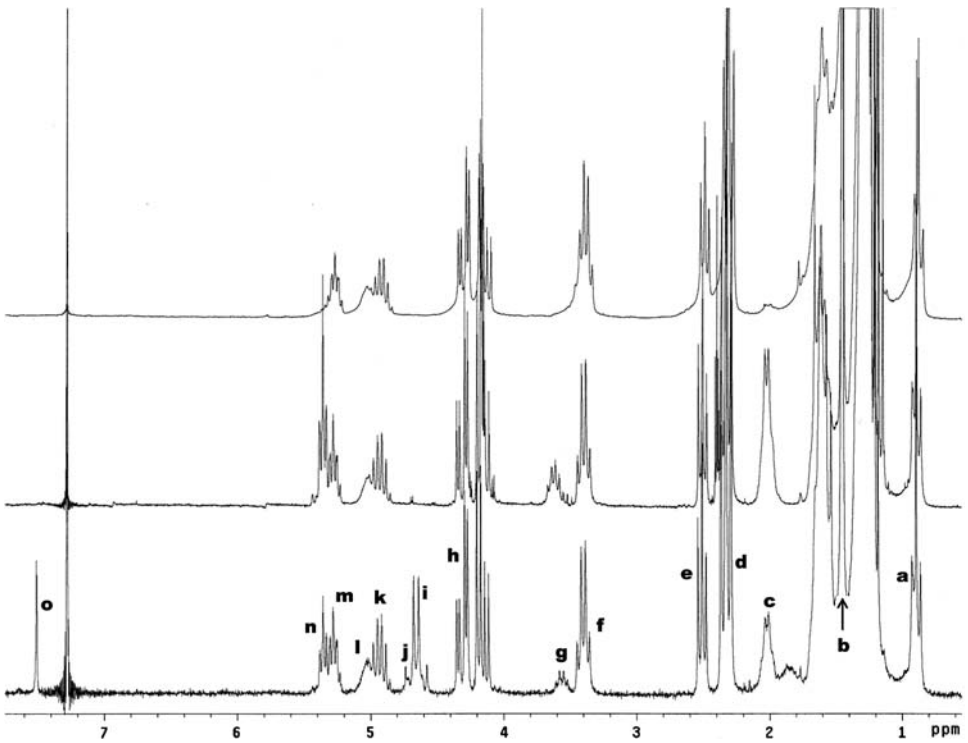


Fig. 2 ^{1}H NMR spectrum of molecule **9** (*bottom*), with peaks labeled according to "Experimental" and Scheme 2, and its two precursors. At the *top* is the azide component; in the *middle* is the alkyne, showing the alkynyl CH as a tight triplet at about 2.4 ppm

a membrane. Molecule **1a**, derivatized at its carboxyl group with an alkyne unit, could also be used to form a glycoside linkage with something other than sophorose at its 17-hydroxyl. A wide range of saccharides would thus be tetherable to a lipid bilayer, or to some other sort of monolayer or solid surface.

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