molecules

MDPI

Article

Chemo-Enzymatic Synthesis of Oligoglycerol Derivatives

Abhishek K. Singh [1], Remi Nguyen [2], Nicolas Galy [2], Rainer Haag [3], Sunil K. Sharma [1,*] and Christophe Len [2,*]

1 Department of Chemistry, University of Delhi, Delhi 110007, India; abhikmcdu@gmail.com
2 Université de Technologie de Compiègne (UTC), CS 60319, Compiègne Cedex 60203, France; remi.nguyen@utc.fr (R.N.); ngaly@laposte.net (N.G.)
3 Institut für Chemie und Biochemie, Freie Universität Berlin, Takustraße 3, Berlin 14195, Germany; rainer.haag@gmx.de
* Correspondence: sk.sharma90@gmail.com (S.K.S.); christophe.len@utc.fr (C.L.); Tel: +33-344-238-82 (C.L.)

Academic Editor: Davide Ravelli
Received: 20 July 2016; Accepted: 5 August 2016; Published: 9 August 2016

Abstract: A cleaner and greener method has been developed and used to synthesize 14 different functionalized oligomer derivatives of glycerol in moderate 29%–39% yields over three steps. After successive regioselective enzymatic acylation of the primary hydroxyl groups, etherification or esterification of the secondary hydroxyl groups and chemoselective enzymatic saponification, the target compounds can efficiently be used as versatile building blocks in organic and supramolecular chemistry.

Keywords: glycerol oligomers; Novozym 435; chemo-enzymatic; regioselectivity; building blocks

1. Introduction

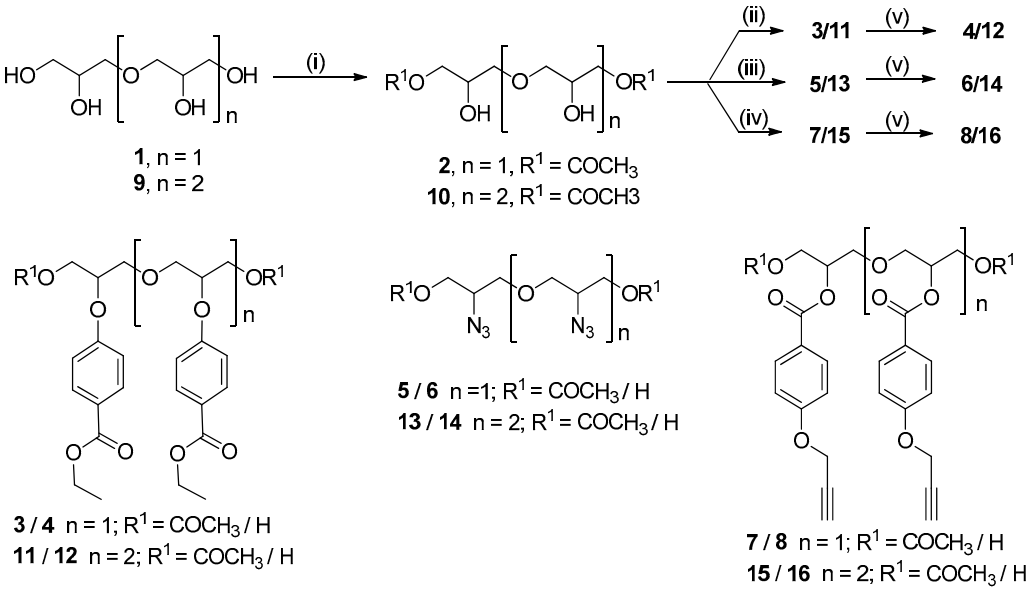
Glycerol is a considered green feedstock due to its bioavailability. An enormous quantity of glycerol is being produced by the oleochemical and biodiesel industry and is used as a base chemical for the production of value added products [1–13]. It has enormous applications in the food industry, pharmaceutical and personal care preparations. Oligomerization of glycerol and the physical and chemical properties of its oligomers have also been well studied. In particular the low molecular weight oligomers such as di-, tri-, and tetraglycerol are more hydrophilic than higher ones and thus have better solubility in polar solvents. These oligomers are used in personal care formulations for their mild humectant properties and ability to enhance fragrance/flavor impact and longevity. Glycerol oligomers also act as plasticizers in PVA films and starch-based biodegradable thermoplastic compositions. The oligomer derivatives have been explored for various applications, e.g., thickners, emulsifiers, antifogging agents, etc. [14–19]. Glycerol oligomers may also be considered as superior building blocks for polymerization or polycondensation reactions in comparison to the monomer as the latter leads to low molecular weight reaction products which have a considerable effect on the properties of the macromolecular compounds. The preparation of polymers based on the conversion of oligomerss into macromolecular compounds is emerging as an interesting line of development in the synthesis of polymers [20,21]. Such a method is associated with the synthesis of oligomers with reactive groups at the ends of the molecules.

In order to provide more extensive data for a wider structure-activity relationship (SAR), analogues having 2-*O*-alkyl and 2-*O*-acyl groups have to be synthesized selectively via full or partial green chemistry. Unfortunately, glycerol and its oligomers have two types of hydroxyl groups having similar pKa and consequently, regioselective differentiation of the primary and secondary hydroxyl groups is difficult. In this regard, a protection-functionalization-deprotection strategy has to be realized.

Our preliminary work reported that an enzymatic method using immobilized *Candida antarctica* lipase (Novozym 435) [22–24] can distinguish the primary and secondary hydroxyl groups in glycerol and polyglycerol moieties to synthesize a wide variety of polymeric and dendritic architectures for biomedical applications [25–29]. Starting from dimers and trimers of glycerol, regio-selective enzymatic protection of the primary hydroxyl groups by acetylation could be envisaged followed by 2-*O*-alkylation or 2-*O*-acylation of the secondary hydroxyl groups, then followed by deprotection of primary hydroxyls. Herein, we report the synthesis of 14 new building blocks based on diglycerol and triglycerol wherein aromatic/azido groups have been incorporated in the secondary carbon via an ether or an ester so as to provide versatility besides facilitating the monitoring of their reactions and product purification. The presence of aromatic moiety may provide the possibility of additional π-π interactions in the macromolecules and thus controlling the aggregation phenomenon and encapsulation behavior. The incorporation of the azido group and an alkynyl group on the other hand provide a site for the 1,3-dipolar cycloaddition reaction under click conditions [30–32].

2. Results

Commercially available diglycerol and triglycerol were subjected to Novozym 435-catalyzed acylation by following the literature procedure [26]. Since the commercially available oligomers are not in pure form and rather are mixtures of glycerol, diglycerol and triglycerol, consequently, a mixture of products is obtained after acylation using Novozym 435 (20 wt %) and vinyl acetate in THF (Scheme 1).



Scheme 1. Synthesis of diglycerol/triglycerol based building blocks. *Reagents and Conditions*: (**i**) Novozym 435, vinyl acetate, THF, 6 h, 30 °C; (**ii**) DIAD, TPP, THF, ethyl,4-hydroxybenzoate, 15 h, 40 °C (**iii**) (a) MsCl, DCM, TEA, 2.5 h, 0 to −5 °C. (b) DMF, NaN_3, 90 °C, 15 h. (**iv**) EDC.HCl, DCM:DMF (4:1), 4-(prop-2-yn-1-yloxy)benzoic acid, 15 h, 30 °C. (**v**) Novozym 435, *n*-butanol, THF, 72 h, 60 °C.

The reaction progress was monitored by TLC (methanol/chloroform, 1:9, *v*/*v*) and on completion of the reaction the desired diacylated product was purified through column chromatography over silica gel using $CHCl_3$/MeOH as eluent It should be noted that compounds **2** and **10** contain two and three stereocenters, respectively. In our hands, both of these compounds were obtained as a mixture of three and seven stereoisomers, respectively and this was confirmed by the observance of the peak multiplicity in the ^{13}C-NMR spectrum of compounds **2** and **10** (see Supplementary Materials Figures S1 and S8). This suggests that the enzymatic conditions produced the protected glycerol

derivatives regioselectively but not stereoselectively. In the ^{1}H-NMR spectrum of compounds **2** and **10**, the methyl protons of the acetyl group appeared at 2.08 ppm, whereas the methylene and methine protons appeared in the range of 3.43 to 4.24 ppm (Figures 1 and 2).

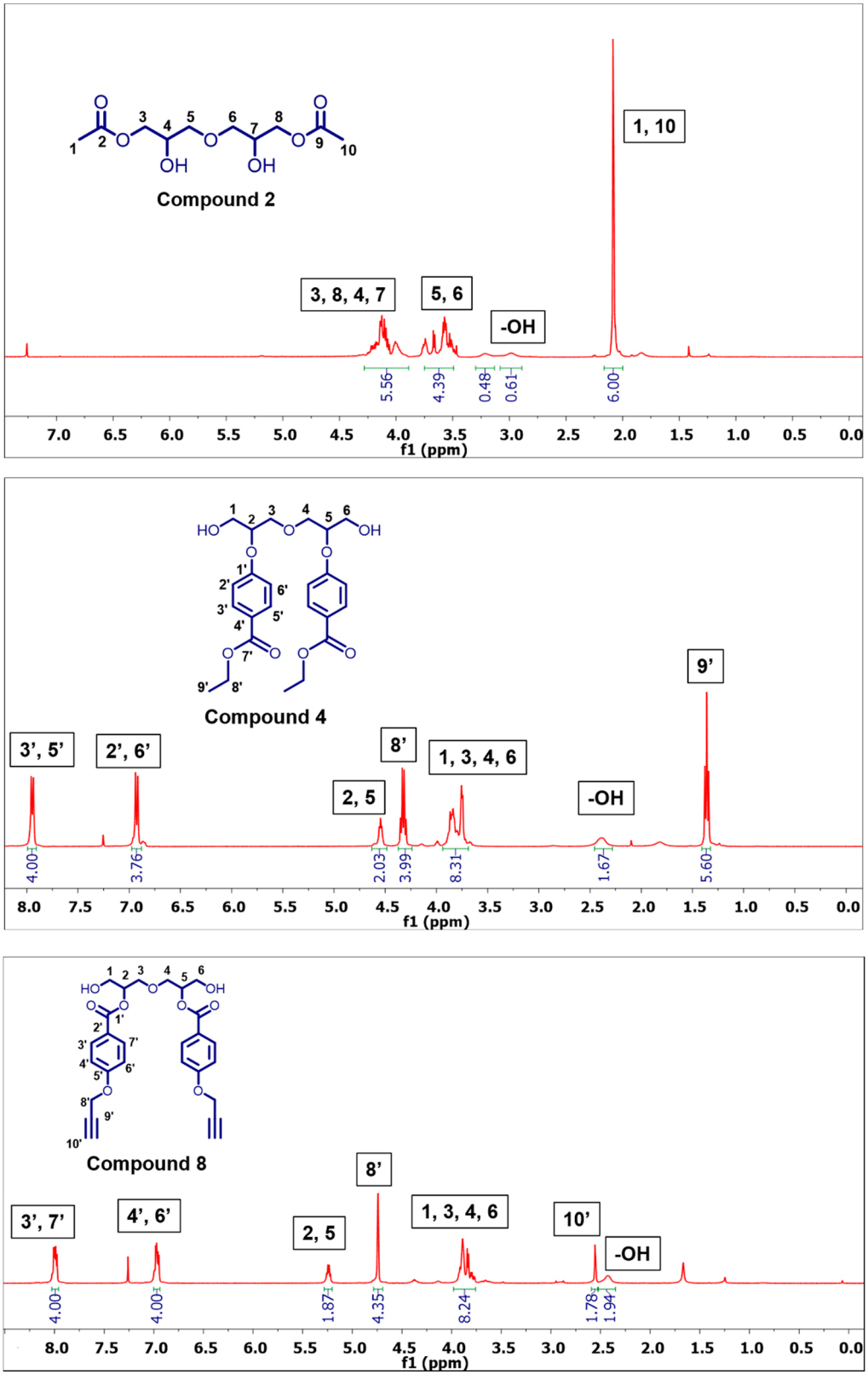


Figure 1. ^{1}H-NMR spectrum of compound **2**, **4**, and **8**.

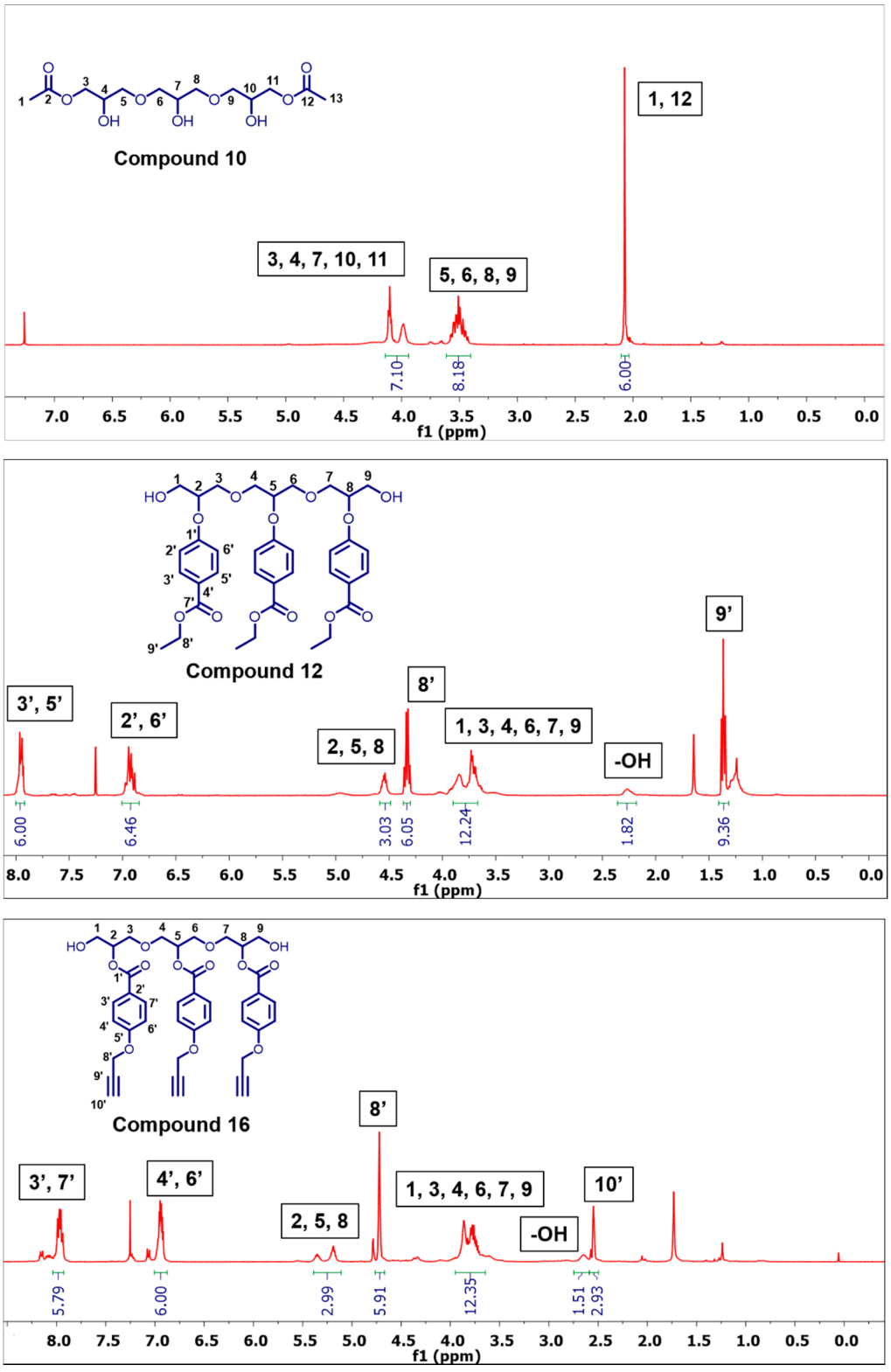


Figure 2. ^{1}H-NMR spectrum of compound **10**, **12** and **16**.

The resulting diacyl oligomers **2** and **10** were further functionalized at the secondary hydroxyl via ether/ester linkage (Scheme 1). The 2-*O*-alkylation of compounds **2** and **10** was realized via Mitsunobu reaction using 4-hydroxybenzoic acid ethyl ester and furnished the two ethers **3** and **11** in 70% and

65% yield, respectively [33]. The 2-*O*-acylation of compounds **2** and **10** using benzoic acid derivative in the presence of EDC gave the corresponding esters **7** and **15** in 75% and 70% yield, respectively [25,34].

Furthermore, the azido group was also incorporated in a stepwise manner i.e., first carrying out mesylation of the secondary hydroxyl group followed by treatment with sodium azide in DMF. The formation of products **5** and **13** was monitored by the appearance of an azide peak in the IR spectra. Our conditions permitted us to produce regioselectively, in the secondary position, the ethers **3** and **11**, esters **7** and **15** and azido derivatives **5** and **13**. Saponification and transesterification were not observed, meaning that the regioselective protection of the primary hydroxyl groups supported the described nucleophilic conditions.

In the next step the resulting diacyl oligomer derivatives **3**, **5**, **7**, **11**, **13**, and **15** were subjected to Novozym 435-catalyzed deacetylation in the presence of excess *n*-butanol in THF [35]. After column chromatography, the target compounds **4**, **6**, **8**, **12**, **14** and **16** were obtained in good yields. A high chemo-selectivity was observed for deacylation of compounds **3**, **7**, **11** and **15**, the aromatic acid ester remained intact and only the selective hydrolysis of aliphatic acid ester was observed. Moreover, in our conditions, no transesterification of the aromatic esters was observed from the secondary hydroxyl group to the primary hydroxyl group. All the compounds were characterized by ^{1}H-, ^{13}C-NMR and HRMS analysis. The methine proton in all of these compounds undergoes a characteristic chemical shift on functionalization i.e., *O*-alkylation of secondary hydroxyl groups in compounds **4**, **8**, **12** and **16** led to a shift from 3.90–4.02 to 4.50–4.55 ppm (Figures 1 and 2). However, on acylation of the secondary hydroxyl group, the corresponding methine proton undergoes a more significant shift to 5.20–5.44 ppm (Figures 1 and 2). The azido compounds **6** and **14** exhibited a characteristic peak for azide group at about 2100 cm^{-1} in the IR spectrum. While in the compounds **8** and **16** the characteristic acetylinic proton was observed at 2.55–2.56 ppm (Figures 1 and 2) in the ^{1}H-NMR spectrum and the acetylinc (-C≡C-) moiety led to the observance of a peak in the rang 2100–2200 cm^{-1} in the IR spectrum.

3. Experimental Section

3.1. General Information

All the compounds were characterized by their physical and spectral data. Infrared spectra were recorded on a Perkin-Elmer FT-IR Model 9 Spectrophotometer (Perkin-Elmer, Singapore). The ^{1}H- and ^{13}C-NMR spectra (400 MHz/100.5 MHz) were recorded on Jeol-400 NMR spectrometer (Jeol, Tokyo, Japan) using TMS as an internal standard. The chemical shift values are measured on δ scale and the coupling constant values (*J*) are reported in Hz. The HRMS data were recorded on Agilent-6530, Q-TOF LCMS (Agilent, Singapore).

The diglycerol and triglycerol were obtained from Solvay Bruxelles (Solvay, Bruxelles, Belgium) and Sigma Aldrich (Saint Louis, MO, USA) respectively. All other chemicals and solvents used were purchased from the Spectrochem Pvt. Ltd. (Mumbay, India) and SD Fine Chemicals Pvt. Ltd. (Mumbay, India). All the solvents were distilled prior to their use. Novozym 435 (immobilized *Candida antarctica* lipase) was purchased from Novo Nordisk A/S, Bagsvaerd, Denmark. Reactions were monitored by pre-coated TLC plates (Merck silica gel 60 F254, Darmstadt, Germany), by visualizing the spot in ceric solution stain and iodine. All the compounds were purified by column chromatography using silica gel (100–200 mesh).

3.2. Synthesis and Characterization

3.2.1. Synthesis of Oxybis(2-hydroxypropan-3,1-diyl) Diacetate (**2**)

In a 250 mL RB flask diglycerol (**1**, 30.1 mmol) was dissolved in THF (150 mL) followed by the addition of Novozym 435 (20 wt % of monomers). After stirring for 10 min vinyl acetate (69.3 mmol, 5.96 g) was added, the reaction mixture was placed in an incubator shaker at 230 rpm for 6 h at 30 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, *v*/*v*).

On completion of the reaction, the enzyme was filtered off and washed with methanol. The organic solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography using $CHCl_3$–MeOH to give the desired compound **2** as a viscous liquid (75%); IR (neat) ν_{max}: 3778, 3698, 2983, 1743, 1712 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.24–3.97 (m, 6H, H-3, H-4, H-7, H-8), 3.67–3.53 (4H, m, H-5, H-6), 3.22 (br s, 1H, OH), 3.00 (br s, 1H, OH), 2.08 (s, 6H, H-1, H-10) ppm; ^{13}C-NMR ($CDCl_3$): δ 171 (C-2, C-9), 72 (C-5, C-6), 68 (C-4, C-7), 65 (C-3, C-8), 20 (C-1, C-10) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{10}H_{18}O_7$: 250.1053; found $[M + Na]^+$: 273.0946.

3.2.2. Synthesis of ((2-Hydroxypropane-1,3-diyl)bis(oxy))bis(2-hydroxypropane-3,1-diyl) Diacetate (**10**)

In a 250 mL RB flask triglycerol (**9**, 30.1 mmol) was dissolved in THF (150 mL) followed by the addition of Novozym 435 (20 wt % of monomers). After stirring for 10 min vinyl acetate (69.3 mmol, 5.96 g) was added, the reaction mixture was placed in an incubator shaker at 230 rpm for 6 h at 30 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, v/v). On completion of the reaction, the enzyme was filtered off and washed with methanol. The organic solvent was evaporated under reduced pressure. The obtained crude product was purified by column chromatography using $CHCl_3$-MeOH to give the desired compound **10** as a viscous liquid (70%); IR (neat) ν_{max}: 3399, 2880, 1724, 1694 1443 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.14–4.08 (m, 4H, H-3, H-11), 4.02–3.93 (m, 3H, H-4, H-7, H-10), 3.58–3.43 (m, 8H, H-5, H-6, H-8, H-9), 2.08 (s, 6H, H-1, H-13) ppm; ^{13}C-NMR ($CDCl_3$): δ 171 (C-2, C-12), 72 (C-5, C-6, C-8, C-9), 69, 68 (C-4, C-7, C-10), 65 (C-3, C-11), 21 (C-1, C-13) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{13}H_{24}O_9$: 324.1420; found $[M + Na]^+$: 347.1909.

3.2.3. Synthesis of Diethyl 4,4′-((Oxybis(1-acetoxypropane-3,2-diyl))bis(oxy))dibenzoate (**3**)

To a stirred solution of compound **2** (4.0 mmol), ethyl,4-hydroxybenzoate (8.4 mmol, 1. 39 g) and triphenylphosphine (12.0 mmol, 3.15 g) in THF (20 mL), DIAD (10 mmol, 2.02 g) in THF (5 mL) was added dropwise. The reaction mixture was stirred for 15 h at 40 °C. The progress of the reaction was monitored by TLC (ethyl acetate–petroleum ether, 1:1, v/v). On completion of the reaction, the reaction mixture was concentrated under reduced pressure and the desired compound was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuuo. The obtained crude product was purified through column chromatography using petroleum ether-ethyl acetate to give the desired compound **3** as a viscous liquid (70%); IR (neat) ν_{max}: 2981, 1740, 1708, 1603, 1506 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.97–7.94 (m, 4H, H-3′, H-5′), 6.95–6.93 (m, 4H, H-2′, H-6′), 4.69–4.64 (m, 2H, H-4, H-7), 4.34–4.28 (m, 8H, H-3, H-8, H-8′), 3.77–3.70 (m, 4H, H-5, H-6), 2.02 (s, 6H, H-1, H-10), 1.36 (t, 6H, J = 8.0, H-9′) ppm; ^{13}C-NMR ($CDCl_3$): δ 170 (C-2, C-9), 166 (C-7′), 161 (C-1′), 131 (C-3′, C-5′), 123 (C-4′), 115 (C-2′, C-6′), 74 (C-4, C-7), 70 (C-5, C-6), 63 (C-3, C-8), 60 (C-8′), 20 (C-1, C-10), 14 (C-9′) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{28}H_{34}O_{11}$: 546.2101; found $[M + H]^+$: 547.2162.

3.2.4. Synthesis of Diethyl 4,4′-((9-(4-(Ethoxycarbonyl)phenoxy)-2,16-dioxo-3,7,11,15-tetraoxahepta-decane-5,13-diyl)bis(oxy))dibenzoate (**11**)

To a stirred solution of compound **10** (4.0 mmol), ethyl,4-hydroxybenzoate (8.4 mmol, 1.39 g) and triphenylphosphine (12.0 mmol, 3.15 g) in THF (20 mL), DIAD (10 mmol, 2.02 g) in THF (5 mL) was added drop wise. The reaction mixture was stirred for 15 h at 40 °C. The progress of the reaction was monitored by TLC (ethyl acetate–petroleum ether, 1:1, v/v). On completion of the reaction, the reaction mixture was concentrated under reduced pressure and the desired compound was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was dried over anhydrous Na_2SO_4 and concentrated in vacuuo. The obtained crude product was purified through column chromatography using petroleum ether–ethyl acetate to give the desired compound 11 as a viscous liquid (65%); IR (neat) ν_{max}: 2992, 1741, 1707, 1603, 1507 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.97–7.90 (m, 6H, H-3′, H-5′), 6.95-6.85 (m, 6H, H-2′,

H-6′), 4.67–4.54 (m, 3H, H-4, H-7, H-10), 4.36–4.31 (m, 6H, H-8′), 4.28–4.25 (m, 4H, H-3, H-11), 3.76–3.66 (m, 8H, H-5, H-6, H-8, H-9), 2.06, 2.02 (s, 6H, H-1, H-13), 1.39–1.35 (m, 9H, H-9′) ppm; ^{13}C-NMR ($CDCl_3$): δ 170 (C-2, C-12), 166 (C-7′), 161 (C-1′), 131 (C-3′, C-5′), 123 (C-4′), 115 (C-2′, C-6′), 76, 74 (C-4, C-7, C-10), 70 (C-5, C-6, C-8, C-9), 63 (C-3, C-11), 60 (C-8′), 20 (C-1, C-13), 14 (C-9′) ppm; HRMS (positive, acetonitrile): m/z calcd for $C_{40}H_{48}O_{15}$: 768.2993; found $[M + NH_4]^+$: 786.3316.

3.2.5. Synthesis of Oxybis(2-azidopropane-3,1-diyl) Diacetate (**5**)

A solution of compound **2** (4.0 mmol) in DCM (30 mL) was cooled under nitrogen atmosphere over ice bath, triethylamine (16.0 mmol, 1.62 g) and methanesulfonyl chloride (12 mmol, 1.37 g) were then added with maintaining the temperature of the reaction mixture at 0 °C. The solution was then stirred at 30 °C for 2.5 h, the progress of the reaction was monitored by TLC (methanol–chloroform, 1:19, v/v). On completion of the reaction, the salt was filtered and the solvent evaporated under reduced pressure. To the mesylated product (4 mmol) so obtained, sodium azide (24 mmol, 1.56 g) and DMF (30 mL) were added, the reaction mixture was heated at 90 °C for 15 h. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:19, v/v). On completion of the reaction, DMF was removed under reduced pressure, the residue obtained was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was dried over anhydrous sodium sulphate followed by evaporation of solvent. The crude product was purified by column chromatography using petroleum ether-ethyl acetate to give the desired compound **5** as a viscous liquid (75%); IR (neat) ν_{max}: 2922, 2875, 2094, 1739, 1449 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.24–4.06 (m, 4H, H-3, H-8), 3.78-3.67 (m, 6H, H-4, H-5, H-6, H-7), 2.09, 2.08 (s, 6H, H-1, H-10) ppm; ^{13}C-NMR ($CDCl_3$): δ 170 (C-2, C-9), 71 (C-5, C-6), 63 (C-3, C-8), 59 (C-4, C-7), 20 (C-1, C-10) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{10}H_{16}N_6O_5$: 300.1182; found $[M + H]^+$: 301.1409.

3.2.6. Synthesis of ((2-Azidopropane-1,3-diyl)bis(oxy))bis(2-azidopropane-3,1-diyl) Diacetate (**13**)

A solution of compound **10** (4.0 mmol) in DCM (30 mL) was cooled under nitrogen atmosphere over ice bath, triethylamine (16.0 mmol, 1.62 g) and methanesulfonyl chloride (12 mmol, 1.37 g) were then added with maintaining the temperature of the reaction mixture at 0 °C. The solution was then stirred at 30 °C for 2.5 h, the progress of the reaction was monitored by TLC (methanol–chloroform, 1:19, v/v). On completion of the reaction, the salt was filtered and the solvent evaporated under reduced pressure. To the mesylated product (4 mmol) so obtained, sodium azide (24 mmol, 1.56 g) and DMF (30 mL) were added, the reaction mixture was heated at 90 °C for 15 h. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:19, v/v). On completion of the reaction, DMF was removed under reduced pressure, the residue obtained was extracted with ethyl acetate (3 × 30 mL). The combined organic layer was dried over anhydrous sodium sulphate followed by evaporation of solvent. The crude product was purified by column chromatography using petroleum ether-ethyl acetate to give the desired compound **13** as a viscous liquid (70 %); IR (neat) ν_{max}: 2919, 2101, 1737, 1450 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 4.24–4.09 (m, 4H, H-3, H-11), 3.82–3.49 (m, 11H, H-4, H-5, H-6, H-7, H-8, H-9, H-10), 2.08 (s, 6H, H-1, H-13) ppm; ^{13}C-NMR ($CDCl_3$): δ 170 (C-1, C-13), 71 (C-5, C-6, C-8, C-9,), 70 (C-3, C-11), 63 (C-7), 59 (C-4, C-10), 20 (C-1, C-13) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{10}H_{18}O_7$: 399.1615; found $[M + NH_4]^+$: 417.1971.

3.2.7. Synthesis of Oxybis(1-acetoxypropane-3,2-diyl) bis(4-(prop-2-yn-1-yloxy)benzoate) (**7**)

Compound **2** (4.0 mmol) and 4-(prop-2-yn-1-yloxy)benzoic acid (8.4 mmol) were dissolved in a mixture of DCM and DMF in 4:1 ratio (30 mL). The reaction mixture was stirred at 0 °C, then EDC.HCl (10 mmol, 1.91 g) and DMAP (6 mmol, 0.73 g) were added, the reaction mixture was stirred for 15 h at 40 °C. The progress of the reaction was monitored by TLC (petroleum ether–ethyl acetate, 1:1, v/v). On completion of the reaction, the solvent was removed under reduced pressure and the residue obtained was extracted with ethyl acetate (3 × 30 mL), the organic layer was dried over anhydrous Na_2SO_4 and solvent evaporated under reduced pressure. The resulting crude product

was purified by column chromatography using petroleum ether–zethyl acetate to give the desired compound **7** as a viscous liquid (75%); IR (neat) ν_{max}: 3284, 2932, 2878, 2123, 1712, 1603, 1508 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.98–7.96 (m, 4H, H-3′, H-7′), 6.98–6.95 (m, 4H, H-4′, H-6′), 5.44–5.38 (m, 2H, H-4, H-7), 4.75–4.74 (m, 4H, H-8′), 4.41–4.29 (m, 4H, H-3, H-8), 3.82–3.70 (m, 4H, H-5, H-6), 2.56 (m, H-10′), 2.03 (s, 6H, H-1, H-10) ppm; ^{13}C-NMR ($CDCl_3$): δ 171 (C-2, C-9), 165 (C-1′), 161 (C-5′), 132 (C-3′, C-7′), 122 (C-2′), 114 (C-4′, C-6′), 77 (C-9′), 76 (C-10′), 70 (C-4, C-7), 69 (C-5, C-6), 62 (C-3, C-8), 55 (C-8′), 21 (C-1, C-10) ppm; HRMS (positive, acetonitrile): *m*/*z* calcd. for $C_{30}H_{30}O_{11}$: 566.1788; found $[M + NH_4]^+$: 584.2123.

3.2.8. Synthesis of 2,16-Dioxo-9-((4-(prop-2-yn-1-yloxy)benzoyl)oxy)-3,7,11,15-tetraoxaheptadecane-5,13-diyl bis(4-(prop-2-yn-1-yloxy)benzoate) (**15**)

Compound **10** (4.0 mmol) and 4-(prop-2-yn-1-yloxy)benzoic acid (8.4 mmol) were dissolved in a mixture of DCM and DMF in 4:1 ratio (30 mL). The reaction mixture was stirred at 0 °C, then EDC.HCl (10 mmol, 1.91 g) and DMAP (6 mmol, 0.73 g) were added, the reaction mixture was stirred for 15 h at 40 °C. The progress of the reaction was monitored by TLC (petroleum ether–ethyl acetate, 1:1, *v*/*v*). On completion of the reaction, the solvent was removed under reduced pressure and the residue obtained was extracted with ethyl acetate (3 × 30 mL), the organic layer was dried over anhydrous Na_2SO_4 and solvent evaporated under reduced pressure. The resulting crude product was purified by column chromatography using petroleum ether–ethyl acetate to give the desired compound **15** as a viscous liquid (70%); IR (neat) ν_{max}: 3286, 2923, 2122, 1714, 1604, 1507 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.18–7.95 (m, 6H, H-3′, H-7′), 7.26–6.94 (m, 6H, H-4′, H-6′), 5.39–5.24 (m, 3H, H-4, H-7, H-10), 4.80–4.73 (m, 6H, H-8′), 4.37–4.27 (m, 4H, H-3, H-11), 3.90–3.62 (m, 8H, H-5, H-6, H-8, H-9), 2.58–2.50 (m, 3H, H-10′), 2.03 (s, 6H, H-1, H-13) ppm; ^{13}C-NMR ($CDCl_3$): δ 170 (C-2, C-12), 165 (C-1′), 161 (C-5′), 131 (C-3′, C-7′), 123 (C-2′), 114 (C-4′, C-6′), 77 (C-9′), 76 (C-10′), 70 (C-4, C-7, C-10), 70 (C-5, C-6, C-8, C-9), 62 (C-3, C-11), 55 (C-8′) 20 (C-1, C-13) ppm; HRMS (positive, acetonitrile): *m*/*z* calcd. for $C_{43}H_{42}O_{15}$: 798.2524; found $[M + NH_4]^+$: 816.2855.

3.2.9. Synthesis of 4,4′-((Oxybis(1-hydroxypropane-3,2-diyl))bis(oxy))dibenzoate (**4**)

Compound **3** (0.91 mmol) was dissolved in THF (1 mL), then *n*-butanol (1 mL) and Novozym 435 (50 wt % of monomer) were added. The reaction mixture was kept in an incubator shaker at 230 rpm for 72 h at 60 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, *v*/*v*). On completion of the reaction, the enzyme was filtered off and washed with methanol. The filtrate was concentrated under reduced pressure. The crude product so obtained was purified by column chromatography using $CHCl_3$–MeOH to give the desired compound **4** as a viscous liquid (75%); IR (neat) ν_{max}: 3440, 2935, 17033, 1603, 1507 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 7.95 (d, 4H, *J* = 8.0, H-3′, H-5′), 6.93 (d, 4H, *J* = 8.0, H-2′, H-6′), 4.56–4.54 (m, 2H, H-2, H-5), 4.36–4.30 (m, 4H, H-8′), 4.00–4.72 (m, 8H, H-1, H-3, H-4, H-6), 2.39 (br s, 2H, OH), 1.37 (t, 6H, H-9′) ppm; ^{13}C-NMR ($CDCl_3$): δ 166 (C-7′), 161 (C-1′), 131 (C-3′, C-5′), 123 (C-4′), 115 (C-2′, C-6′), 77 (C-2, C-5), 70 (C-3, C-4), 62 (C-1, C-6), 61 (C-8′), 14 (C-9′) ppm; HRMS (positive, acetonitrile): *m*/*z* calcd. for $C_{24}H_{30}O_9$: 462.1890; found $[M + H]^+$: 463.1964.

3.2.10. Synthesis of Diethyl 4,4′-((((2-(4-(ethoxycarbonyl)phenoxy)propane-1,3-diyl)bis(oxy))bis(1-hydroxypropane-3,2-diyl))bis(oxy))dibenzoate (**12**)

Compound **11** (0.91 mmol) was dissolved in THF (1 mL), then *n*-butanol (1 mL) and Novozym 435 (50 wt % of monomer) were added. The reaction mixture was kept in an incubator shaker at 230 rpm for 72 h at 60 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, *v*/*v*). On completion of the reaction, the enzyme was filtered off and washed with methanol. The filtrate was concentrated under reduced pressure. The crude product so obtained was purified by column chromatography using $CHCl_3$–MeOH to give the desired compound **12** as a viscous liquid (65%); IR (neat) ν_{max}: 3465, 2980, 2928, 1702, 1603, 1507 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 25 °C): δ 7.97–7.94 (m, 6H,

H-3′, H-5′), 6.98–6.89 (m, 6H, H-2′, H-6′), 4.58–4.53 (m, 3H, H-2, H-5, H-8), 4.37–4.31 (m, 6H, H-8′), 3.92–3.70 (m, 12H, H-1, H-3, H-4, H-6, H-7, H-9), 2.27 (br s, 2H, OH), 1.39 (m, 9H, H-9′) ppm; ^{13}C-NMR ($CDCl_3$): δ 166 (C-7′), 161 (C-1′), 131 (C-3′, C-5′), 124 (C-4′), 115 (C-2′, C-6′), 75, 70 (C-2, C-5, C-8), 62 (C-3, C-4, C-6, C-7), 62 (C-1, C-9), 60 (C-8′), 14 (C-9′) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{36}H_{44}O_{13}$: 684.2773; found $[M + NH_4]^+$: 702.3113.

3.2.11. Synthesis of 3,3′-Oxybis(2-azidopropan-1-ol) (**6**)

Compound **5** (1.6 mmol) was dissolved in THF (1 mL), then *n*-butanol (1 mL) and Novozym 435 (50 wt % of monomer) were added. The reaction mixture was kept in an incubator shaker at 230 rpm for 72 h at 60 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, v/v). On completion of the reaction, the enzyme was filtered off and washed with methanol. The filtrate was concentrated under reduced pressure. The crude product obtained was purified by column chromatography using $CHCl_3$–MeOH as an eluent to give the desired compound **6** as a viscous liquid (70%); IR (neat) ν_{max}: 3357, 2926, 2978, 2088, 1638, 1464 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.78–3.65 (m, 10H, H-1, H-2, H-3, H-4, H-5, H-6), 2.45 (br s, 2H, OH) ppm; ^{13}C-NMR ($CDCl_3$): δ 71 (C-3, C-4), 62 (C-2, C-5), 62 (C-1, C-6) ppm. HRMS (positive, acetonitrile): m/z calcd. for $C_6H_{12}N_6O_3$: 216.0971; found $[M + H]^+$: 217.1044.

3.2.12. Synthesis of 3,3′-((2-Azidopropane-1,3-diyl)bis(oxy))bis(2-azidopropan-1-ol) (**14**)

Compound **13** (1.6 mmol) was dissolved in THF (1 mL), then *n*-butanol (1 mL) and Novozym 435 (50 wt % of monomer) were added. The reaction mixture was kept in an incubator shaker at 230 rpm for 72 h at 60 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, v/v). On completion of the reaction, the enzyme was filtered off and washed with methanol. The filtrate was concentrated under reduced pressure. The crude product obtained was purified by column chromatography using $CHCl_3$–MeOH as an eluent to give the desired compound **14** as a viscous liquid (60%); IR (neat) ν_{max}: 3390, 2915, 1988, 2105, 1748, 1480 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 3.78–3.55 (m, 15H, H-1–H-9) ppm; ^{13}C-NMR ($CDCl_3$, 25 °C): δ 71 (C-4, C-6), 70 (C-3, C-7), 62 (C-1, C-9), 62, 60 (C-2, C-5, C-8) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_9H_{17}N_9O_4$: 315.14.04; found $[M + H]^+$: 338.1298.

3.2.13. Synthesis of Oxybis(1-hydroxypropane-3,2-diyl) bis(4-(prop-2-yn-1-yloxy)benzoate) (**8**)

Compound **7** (0.88 mmol) was dissolved in THF (1 mL), then *n*-butanol (1 mL) and Novozym 435 (50 wt % of monomer) were added. The reaction mixture was kept in an incubator shaker at 230 rpm for 72 h at 60 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, v/v). On completion of the reaction, the enzyme was filtered and washed with methanol. The filtrate was concentrated under reduced pressure. The obtained crude product was purified by column chromatography using $CHCl_3$–MeOH to give the desired compound **8** as a viscous liquid (70%); IR (neat) ν_{max}: 3459, 3289, 2932, 2880, 2123, 1701, 1603, 1508 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.01–7.98 (m, 4H, H-3′, H-7′), 6.98–6.95 (m, 4H, H-4′, H-6′), 5.26–5.22 (m, 2H, H-2, H-5), 4.74 (m, 4H, H-8′), 3.92–3.73 (m, 8H, H-1, H-3, H-4, H-6), 2.56 (m, 2H, H-10′), 2.43 (br s, 2H, OH) ppm; ^{13}C-NMR ($CDCl_3$): δ 166 (C-1′), 161 (C-5′), 132 (C-3′, C-7′), 123 (C-2′), 114 (C-4′, C-6′), 77 (C-9′), 76 (C-10′), 73 (C-2, C-5), 70 (C-3, C-4), 62 (C-1, C-6), 55 (C-8′) ppm; HRMS (positive, acetonitrile): m/z calcd. for $C_{26}H_{26}O_9$: 482.1577; found $[M + Na]^+$: 505.1459.

3.2.14. Synthesis of ((2-((4-(Prop-2-yn-1-yloxy)benzoyl)oxy)propane-1,3-diyl)bis(oxy))bis(1-hydroxypropane-3,2-diyl) bis(4-(Prop-2-yn-1-yloxy)benzoate) (**16**)

Compound **15** (0.88 mmol) was dissolved in THF (1 mL), then *n*-butanol (1 mL) and Novozym 435 (50 wt % of monomer) were added. The reaction mixture was kept in an incubator shaker at 230 rpm for 72 h at 60 °C. The progress of the reaction was monitored by TLC (methanol–chloroform, 1:9, v/v). On completion of the reaction, the enzyme was filtered and washed with methanol. The filtrate

was concentrated under reduced pressure. The obtained crude product was purified by column chromatography using $CHCl_3$–MeOH to give the desired compound **16** as a viscous liquid (65%); IR (neat) ν_{max}: 3286, 2955, 2878, 2123, 1713, 1603, 1508 cm^{-1}; ^{1}H-NMR ($CDCl_3$): δ 8.17–7.94 (m, 6H, H-3′, H-7′), 7.08–6.94 (m, 6H, H-4′, H-6′), 5.39–5.18 (m, 3H, H-2, H-5, H-8), 4.79–4.73 (m, 6H, H-8′), 3.96–3.67 (m, 12H, H-1, H-3, H-4, H-6, H-7, H-9), 2.66 (br s, 2H, OH), 2.59–2.55 (m, 3H, H-10′) ppm; ^{13}C-NMR ($CDCl_3$): δ 165 (C-1′), 161 (C-5′), 131 (C-3′, C-7′), 123 (C-2′), 114 (C-4′, C-6′), 77 (C-9′), 76 (C-10′), 73 (C-2, C-5, C-8), 70 (C-3, C-4, C-6, C-7), 62 (C-1, C-9), 55 (C-8′) ppm; HRMS (positive, acetonitrile): *m*/*z* calcd. for $C_{39}H_{38}O_{13}$: 714.2303; found $[M + Na]^+$: 737.2198.

4. Conclusions

A series of 13 novel glycerol oligomer derivatives **3**–**8** and **10**–**16** have been synthesized using chemo-enzymatic approach that can be used further for the synthesis of supramolecular architectures. The enzyme catalyzed approach exhibits high chemo- and regioselectivity as the primary hydroxyl group can be selectively acylated in the presence of secondary hydroxyl group. The acetyl groups of the hydroxymethyl of polymers **2** and **10** were stable during the acylation, etherification and azidation. Moreover selective enzymatic deprotection of the primary hydroxyl groups was efficient without saponification and trans-esterification of the ester in the secondary hydroxyl group.

Supplementary Materials: Supplementary materials can be accessed at: http://www.mdpi.com/1420-3049/21/8/1038/s1.

Acknowledgments: The authors acknowledge the Science & Engineering Research Board (DST-SERB), Government of India for financial support.

Author Contributions: All authors contributed equally to this work. Rainer Haag, Sunil K. Sharma and Christophe Len conceived and designed the experiments; Abhishek K. Singh, Remi Nguyen and Nicolas Galy performed the experiments; Abhishek K. Singh, Remi Nguyen and Nicolas Galy analyzed the data; Rainer Haag, Sunil K. Sharma and Christophe Len contributed reagents/materials/analysis tools; Rainer Haag, Sunil K. Sharma and Christophe Len wrote the paper."

Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations

The following abbreviations are used in this manuscript:

DMF	*N*,*N*-dimethylformamide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
FT-IR	Fourier transform infrared
HRMS	high-resolution mass spectrometry
IR	infrared
MeOH	methanol
NMR	nuclear magnetic resonance
pKa	acid dissociation constant
ppm	parts per million
PVA	polyvinyl acetate
Q-TOF LCMS	quadrupoletime of flight liquid chromatography mass spectrometry
RB	round-bottom
rpm	round per min
SAR	structure-activity relationship
THF	tetrahydrofuran
TLC	thin layer chromatography
TMS	tetramethylsilane

References

1. Katryniok, B.; Kimura, H.; Skrzynska, E.; Girardon, J.; Fongarland, P.; Capron, M.; Ducoulombier, R.; Mimura, N.; Paul, S.; Dumeignil, F. Selective catalytic oxidation of glycerol: perspective for high value chemicals. *Green Chem.* **2011**, *13*, 1960–1979. [CrossRef]

2. Cintas, P.; Tagliapietra, S.; Gaudino, E.C.; Palmisano, G.; Cravotto, G. Glycerol: A solvent and a building block of choice for microwave and ultrasound irradiation procedure. *Green Chem.* **2014**, *16*, 1056–1065. [CrossRef]
3. Behr, A.; Eilting, J.; Irawadi, K.; Leschinski, J.; Lindner, F. Improved utilization of renewable ressources: New important derivatives of glycerol. *Green Chem.* **2008**, *10*, 13–30. [CrossRef]
4. García, J.; Marín, H.; Pires, E. Glycerol based solvents: Synthesis, properties and applications. *Green Chem.* **2014**, *16*, 1007–1033. [CrossRef]
5. Diaz-Alvarez, A.E.; Francos, J.; Lastra-Barreira, B.; Crochet, P.; Cadierno, V. Glycerol and derived solvents: New sustainable reaction media for organic synthesis. *Chem. Commun.* **2011**, *47*, 6208–6227. [CrossRef] [PubMed]
6. Len, C.; Merlot, A.S.; Postel, D.; Ronco, G.; Villa, P.; Goubert, C.; Jeufrault, E.; Mathon, B.; Simon, H. Synthesis and antifungal activity of novel bis (dithiocarbamate) derivatives of glycerol. *J. Agric. Food Chem.* **1996**, *44*, 2856–2858. [CrossRef]
7. Len, C.; Postel, D.; Ronco, G.; Villa, P.; Goubert, C.; Jeufrault, E.; Mathon, B.; Simon, H. Synthesis of carbamic esters derivatives of itols: Antifungal activity against various crop diseases. *J. Agric. Food Chem.* **1997**, *45*, 3–6. [CrossRef]
8. Rafin, C.; Veignie, E.; Sancholle, M.; Postel, D.; Len, C.; Villa, P.; Ronco, G. Synthesis and antifungal activity of novel bisdithiocarbamate derivatives of carbohydrates against *Fusarium oxysporum* f. sp. *Lini*. *J. Agric. Food Chem.* **2000**, *48*, 5283–5287. [CrossRef] [PubMed]
9. DeSousa, R.; Thurier, C.; Len, C.; Pouilloux, Y.; Barrault, J.; Jerome, F. Regioselective functionalization of glycerol with a dithiocarbamate moiety: An environmentally friendly route to safer fungicides. *Green Chem.* **2011**, *13*, 1129–1132. [CrossRef]
10. Saggadi, H.; Luart, D.; Thiebault, N.; Polaert, I.; Estel, L.; Len, C. Toward the synthesis of 6-hydroxyquinoline starting from glycerol via improved microwave-assisted modified Skraup reaction. *Catal. Commun.* **2014**, *44*, 15–18. [CrossRef]
11. Len, C.; Luque, R. Continuous flow transformations of glycerol to valuable products: An overview. *Sustain. Chem. Process.* **2014**, *2*. [CrossRef]
12. Saggadi, H.; Luart, D.; Thiebault, N.; Polaert, I.; Estel, L.; Len, C. Quinoline and phenanthroline preparation starting from glycerol via improved microwave-assisted modified Skraup reaction. *RSC Adv.* **2014**, *4*, 21456–21464. [CrossRef]
13. Saggadi, H.; Polaert, I.; Luart, D.; Len, C.; Estel, L. Microwaves under pressure for the continuous production of quinolone from glycerol. *Catal. Today* **2015**, *255*, 66–74. [CrossRef]
14. Zhou, C.H.; Beltramini, J.N.; Fana, Y.X.; Lu, G.Q. Chemoselective catalytic conversion of glycerol as a biorenewable source to valuable commodity chemicals. *Chem. Soc. Rev.* **2008**, *37*, 527–549. [CrossRef] [PubMed]
15. Martin, A.; Richter, M. Oligomerization of glycerol: A critical review. *Eur. J. Lipid Sci. Technol.* **2011**, *113*, 100–117. [CrossRef]
16. Shi, Y.; Dayoub, W.; Chen, G.R.; Lemaire, M. Selective synthesis of 1-*O*-alkyl glycerol and diglycerol ethers by reductive alkylation of alcohols. *Green Chem.* **2010**, *12*, 2189–2195. [CrossRef]
17. Cassel, S.; Debaig, C.; Benvegnu, T.; Chaimbault, P.; Lafosse, M.; Plusquellec, D.; Rollin, P. Original synthesis of linear, branched and cyclic oligoglycerol standards. *Eur. J. Org. Chem.* **2001**, 875–896. [CrossRef]
18. Sutter, M.; Dayoub, W.; Métay, E.; Raoul, Y.; Lemaire, M. 1-*O*-Alkyl (di)glycerol ethers synthesis from methyl esters and triglycerides by two pathways: catalytic reductive alkylation and transesterification/reduction. *Green Chem.* **2013**, *15*, 786–797. [CrossRef]
19. Katryniok, B.; Paul, S.; Baca, V.; Reye, P. Glycerol dehydration to acrolein in the context of new uses of glycerol. *Green Chem.* **2010**, *12*, 2079–2098. [CrossRef]
20. Urata, K. Unique block molecules based on glycerol skeleton as C-3 building blocks for liquid crystals formation by self-assembly and their future potential for the "nano-chemistry". *Eur. J. Lipid Sci. Technol.* **2003**, *105*, 542–556. [CrossRef]
21. Sonnati, M.O.; Amigoni, S.; Taffin de Givenchy, E.P.; Darmanin, T.; Chouletb, O.; Guittard, F. Glycerol carbonate as a versatile building block for tomorrow: Synthesis, reactivity, properties and applications. *Green Chem.* **2013**, *15*, 283–306. [CrossRef]

22. Kirk, O.; Christensen, M.W. Lipases from *Candida antartica*: Unique biocatalysts from a unique origin. *Org. Proc. Res. Dev.* **2002**, *6*, 446–451. [CrossRef]
23. Anderson, E.M.; Larsson, K.M.; Krik, O. One biocatalyst-many applications: The use of *Candida antartica* B-lipase in organic synthesis. *Biocatal. Biotransform.* **1998**, *16*, 181–204. [CrossRef]
24. Krik, O.; Bjorkling, F.; Godtfredsen, S.E.; Larsen, T.O. Fatty acid specificity in lipase-catalyzed synthesis of glucoside esters. *Biocatalysis* **1992**, *6*, 127–134. [CrossRef]
25. Gupta, S.; Jalal, S.; Kumar, S.; Haag, R.; Sharma, S.K. A simple and convenient chemoenzymatic approach for the synthesis of valuable triacylglycerol-based dendritic building blocks. *Indian J. Chem.* **2012**, *51B*, 1376–1387.
26. Gupta, S.; Schade, B.; Kumar, S.; Böttcher, C.; Sharma, S.K.; Haag, R. Dendronized multiamphiphilic polymers as non-ionic nanotransporters for biomedical applications: Synthesis, aggregation behavior and transport properties. *Small* **2013**, *9*, 894–904. [CrossRef] [PubMed]
27. Kumari, M.; Gupta, S.; Böttcher, C.; Khandare, J.; Sharma, S.K.; Haag, R. Dendronized multifunctional amphiphilic polymers as efficient nanocarriers for biomedical applications. *Macromol. Rapid Commun.* **2015**, *36*, 254–251. [CrossRef] [PubMed]
28. Kumari, M.; Billamboz, M.; Leonard, E.; Len, C.; Böttcher, C.; Prasad, A.K.; Haag, R.; Sharma, S.K. Self-assembly, photoresponsive behavior and transport potential of azobenzene grafted dendronized polymeric amphiphiles. *RSC Adv.* **2015**, *5*, 48301–48310. [CrossRef]
29. Kumar, S.; Achazi, K.; Böttcher, C.; Licha, K.; Haag, R.; Sharma, S.K. Encapsulation and cellular internalization of cyanine dye using amphiphilic dendronized polymers. *Eur. Polym. J.* **2015**, *69*, 416–428. [CrossRef]
30. Thirumurugan, P.; Matosiuk, D.; Jozwiak, K. Click chemistry for drug development and diverse chemical-biology applications. *Chem. Rev.* **2013**, *113*, 4905–4979. [CrossRef] [PubMed]
31. Legros, V.; Vanhaverbecke, C.; Souard, F.; Len, C.; Desire, J. β-Cyclodextrin-glycerol dimers: Synthesis and NMR conformational analysis. *Eur. J. Org. Chem.* **2013**, *2013*, 2583–2590. [CrossRef]
32. Espeel, P.; du Prez, F.E. "Click"-inspired chemistry in macromolecular science: Matching recent progress and user expectations. *Macromolecules* **2015**, *48*, 2–14. [CrossRef]
33. Saku, O.; Ishida, H.; Atsumi, E.; Sugimoto, Y.; Kodaira, H.; Kato, Y.; Shirakura, S.; Nakasato, Y. Discovery of novel 5,5-diarylpentadienamides as orally available transient receptor potential vanilloid 1 (TRPV1) antagonists. *J. Med. Chem.* **2012**, *55*, 3436–3451. [CrossRef] [PubMed]
34. Zhu, Y.Y.; Yin, T.T.; Li, X.L.; Su, M.; Xue, Y.X.; Yu, Z.P.; Liu, N.; Yin, J.; Wu, Z.Q. Chiroptical and thermotropic properties of helical styrenic polymers: effect of achiral group. *Macromolecules* **2014**, *47*, 7021–7029. [CrossRef]
35. Sharma, D.; Khandelwal, A.; Sharma, R.K.; Bhatia, S.; Reddy, L.C.; Olsen, C.E.; Wengel, J.; Parmar, V.S.; Prasad, A.K. Synthesis and regioselective deacylation studies on peracylated 2′-azido arabino- and ribo-thymine nucleosides: towards 5′-*O*,2′-*N*-linked oligonucleotides. *Indian J. Chem.* **2009**, *48B*, 1712–1720. [CrossRef]

Sample Availability: Samples of the compounds are not available from the authors.