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Chemical Biology
LETTERS

An economic and efficient synthesis of acid-labile glycerol based β-thiopropionate esters for potential application in drug delivery

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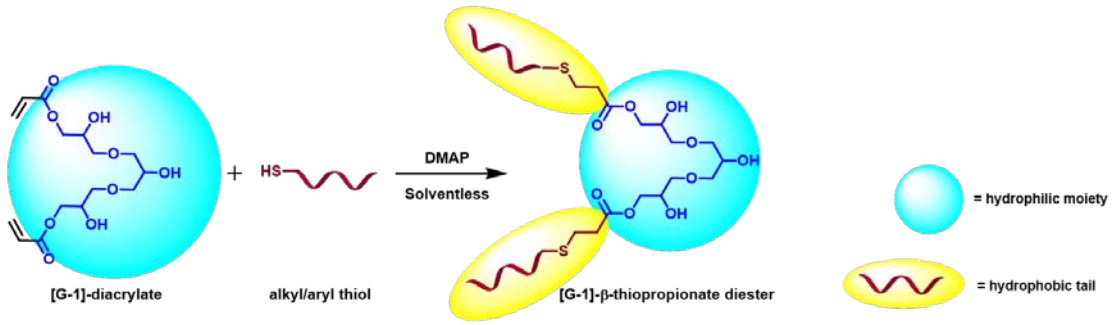
ABSTRACT



Acid labile linkers have been used widely for various biomedical applications with preferential applications in drug delivery. In this report, we have synthesized, glycerol based β-thiopropionate esters having an acid-labile β-thiopropionate linker with Michael addition reaction between hydrophobic thiol and hydrophilic tri-glycerol diacrylate. The solvent free reaction and purification by simply solvent extraction instead of any sophisticated chromatographic techniques provide an upper edge for their application in biomedical or other fields. These β-thiopropionate esters can potentially be used for the delivery and release of hydrophobic drugs at acidic sites particularly in cancer cells.

Keywords: β-thiopropionate, Acid-labile, pH-sensitive, Michael addition reaction

INTRODUCTION

The stimuli-responsive supramolecular assemblies of amphiphilic molecules such as vesicles and micelles are prominent moieties in the field of drug delivery systems because of their extensive explorations towards various possible applications. Among these moieties, the polymeric micelles have considerably evolved over surfactant micelles as they aggregates at very low concentration, have high thermodynamic stability[1,2] and have the ability to respond to the physiochemical changes such as pH, light, redox state of medium, temperature, etc.[3-6] Self-assembled polymeric micelles trap hydrophobic drugs in the hydrophobic core of micelles (non-covalent entrap)[7] or drug molecules can be covalently bonded[8] for their delivery. Recently, degradation of supramolecular aggregates in the presence of stimuli is being studied, however, among all stimuli responsive conjugates, the pH-responsive disassembly of supramolecular aggregates has become very significant in the field of biology.[9] There are a number of studies on pH-sensitive micelles constructs, and commonly employed acid-labile functional groups in such micelles include ketals, acetal, hydrazone, cis-acotinyl and orthoesters.[3] Most of these functional groups are acid sensitive, hence they cleaves rapidly *i.e.* their cleavage rate vary from a few minutes to hours. For example, Zhang *et al*. trapped pyrene (a hydrophobic guest) inside the alkynylated surfactant micelles using click chemistry, and subsequently, pyrene got released rapidly as soon as the micelles were disintegrated.[3]

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Only a few linkers have prospect for pH selective disassembly of polymeric aggregates in a continued manner. β-thiopropionate is one such acid-labile functional group which undergo hydrolysis at slow rate, in contrast to the rapidly cleaved acid-labile groups.[10] Therefore, this functional group is suitable for controlled release of payload or drug. For example, Schoenmakers *et al*. coupled paclitaxel drug to a poly ethylene glycol macromonomer using a hydrolysable linker, *via* Michael addition between a thiol and an acrylamide and it was found that by varying the size of the linker length from 3-sulfanylpropionyl to 4-sulfanylpropionyl half-life time of drug release could be raised from 4.2 to 14 days.[11] Another significant benefit of β-thiopropionate linker is that it can be produced with ease by simple facile thiol acrylate Michael addition reaction based on click chemistry in presence of a catalyst.[12-13] This method of effortless synthesis was initiated by Sharpless and colleagues in 2000.[14] Jones *et al*. reported the synthesis of PEGylated peptides by using the thio-ene click approach.[15] Further, Dan *et al*. studied the aggregation property and pH-responsive disassembly of the aggregates of the hydrophilic and hydrophobic segments of a PEG based surfactant connected by a β-Thiopropionate linker. They have highlighted the utility of the simple thiol-acrylate Michael reaction in terms of generating a pH-responsive functional group.[10] Oishi *et al.* achieved enhanced gene silencing activity in heptoma cells by fabricating lactosylated-PEG-siRNA conjugates having acid labile β-thiopropionate linkage into polyion complex micelles.[16]

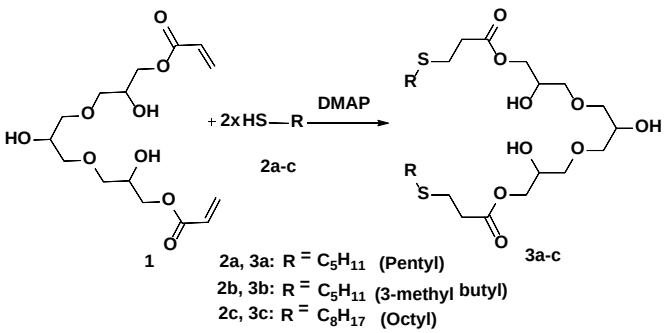
The broad significance of β-thiopropionate motivated us to synthesize dendritic esters by using this linker. The use of this linker has improved targeted drug delivery and reduced the various side effects in rapid drug delivery (such as inadequate stability, less bioavailability and short half-life), and also further, we have used a non-toxic starting material[17] for synthesis of a new class of coumpounds. Glycerol is believed to be a green feedstock because of its bioavailibilty.[18-19] Glycerol oligomers *i.e.* triglycerol and its derivatives have been explored for abundant applications *e.g*. emulsifier, thickners and antifogging agents.[20] This study is focused on synthesis of β-thiopropionate linker based dendritic esters that differ in size or shape of hydrophobic tail by using different thiol compounds. Here in, we have designed an economic and an efficient synthesis for a novel series of five [G-1]-β-thiopropionate diesters compounds. All the synthesized compounds have been well characterised by using modern spectroscopic techniques like FT-IR, ^{1}H and ^{13}C NMR. Modern experimental techniques including DEPT, HMQC and HMBC have been used to better understand the spectral properties of these compounds. These synthesized compounds are believed to open up various exciting possibilities in therapeutic and biomedical applications.

Result and Discussion

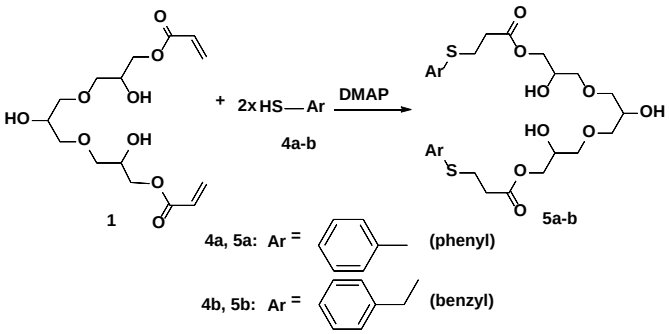
The synthesis of a new class of compounds was carried out in neat conditions *i.e.* solventless synthesis by exploring the Michael addition reaction of different alkyl and aryl thiols on [G-1]-diacrylate dendron. The purification was carried out *via* solvent extraction without the use of chromatographic techniques. The synthetic and purification protocols were very simple and effective, and the products were obtained in pure form with high yields. The products obtained may open up new dimensions to varied applications in biomedical field for drug encapsulation and release *via* steady disassembly of these architectures.

The commercially available synthon (2-Hydroxy-1,3-propanediyl) bis (oxy-2-hydroxy-3,1-propanediyl) bisacrylate (or [G-1]-diacrylate, **1**), is a first generation glycerol dendron with highly hydrophilic hydroxyl groups. Michael addition reaction on two acryloyl functional groups of [G-1] dendron *via* neat reaction conditions and using other commercially available synthons (alkyl and aryl thiols) with variartion in shape and size of chain lengths to introduce different levels of hydrophobicity for the synthesis of these new dendritic architectures.

DMAP catalyzed Michael addition reaction of alkyl thiols (**2a-c**) and aryl thiols (**4a-b**) with [G-1]-diacrylate (**1**) was carried out under solventless conditions by stirring at room temperature for 24 h under argon atmosphere. The completion of the reactions were monitored *via* TLC and the products were purified *via* solvent extraction. The crude product was first washed with hexane and then dissolved in chloroform and washed three times with water. The organic layer was then dried over Na_2SO_4 and the solvent was removed under vacuum to obtain the final products with high purity and significant yields (75-80%). A novel series of five [G-1]-β-thiopropionate diester were thus synthesized by varying the hydrophobic tail by using different thiols *via* **Scheme 1** and **2**.



Scheme 1: Synthesis of β- thiopropionate ester of [G-1] dendron with aliphatic hydrophobic groups



Scheme 2: Synthesis of β-thiopropionate ester of [G-1] dendron with aromatic hydrophobic groups

The final products were confirmed by their spectral data analysis (IR, ^{1}H-NMR and ^{13}C-NMR). The representative compound **3c** was characterized based on its IR, ^{1}H-NMR, ^{13}C-NMR, DEPT-135, HSQC and HMBC spectras. In the IR spectra the characteristic disappearance of peaks in the region of 1630-1615 cm^{-1} for the double bond of acryloyl functional groups confirmed the Michael addition reaction on the double bond. Further, the characteristic presence of aliphatic protons adjacent to Sulphur atom and carbonyl group in the region of δ 2.51–2.80 ppm in the ^{1}H-NMR along with the presence of protons corresponding to [G-1] dendron from δ 3.63-4.00 ppm confirmed the addition of octane thiol and also the absence of ethylene proton at δ 6.39-6.35 ppm confirmed the addition of octane thiol (**2c**) to [G-1]-diacrylate (**1**) (**Figure S9**, c.f. SI). The proposed constitution was further confirmed by the comparison of proton-decoupled ^{13}C-NMR spectrum with the DEPT-135 spectra (**Figure 1**). The positive peaks at δ 14.21 ppm and δ 68.79 ppm corresponding to the terminal methyl carbon of octane thiol and methine carbons of [G-1] dendron, and all other methylene carbons appearing as negative peaks, clearly indicated the successful Michael addition reaction. Absence of ethylene carbon peaks at δ 130.2 and 128.2 ppm indicates the addition of octane thiol to [G-1]-diacrylate. The HSQC (**Figure 2**) and HMBC (**Figure 3**) spectra also confirmed and supported the above facts by giving the proton-carbon single bond correlations, and proton-carbon multiple bond correlations respectively. As the HMBC gives correlation between carbon and proton which are separated by two or three bonds and direct one-bond relation is suppressed. The HMBC spectrum (**Figure 3**) gives information that the peaks encircled shows three bond correlation between C-7 and H-9 and two bond corerelation between C-8 and H-9. All other compouds with aliphatic hydrophobic tails (**3a-b**) were unambiguously characterized on the basis of their spectral data (**Figure S2-S10**, c.f. SI) as for the compound **3c**. Further, two aryl functionalized compounds were prepared using thio phenol (**4a**) and benzyl mercaptum (**4b**) to vary the hydrophobic part under similar reaction conditions. The structure of these two compounds were further well established *via* IR, ^{1}H and ^{13}C NMR spectrum (**Figure S11-S16**, c.f. SI). In the IR spectra of compound **5a** (**Figure S11**, c.f. SI), the characteristic disappearance of peaks in the region of 1630-1615 cm^{-1} for the double bond of acryloyl functional groups and the appearance of peaks in the region from 1600-1585 cm^{-1} and 1500-1400 cm^{-1} for the C-C stretching of aromatic ring and peaks at 738 cm^{-1} and 691 cm^{-1} for out of plane bands confirms the Michael addition of thio phenol (**4a**) to [G-1]-diacrylate (**1**). In the ^{1}H NMR (**Figure S12**, c.f. SI), the characteristic peaks for 10 protons of the two aromatic rings appeared at δ 7.35-7.20 ppm along with the [G-1] dendron's characteristic peaks, confirming the formation of product **5a**. In the ^{13}C NMR spectrum (**Figure S13**, c.f. SI), the peaks at δ 126.6-135.1 ppm confirmed the aromatic carbons, while the carbonyl carbon appeared at δ 171.84 ppm. These peaks for assigned for particular protons and carbons confirmed the structure of the synthesized compounds with further assured by the corelational spectrum plots for the compound **3c**.

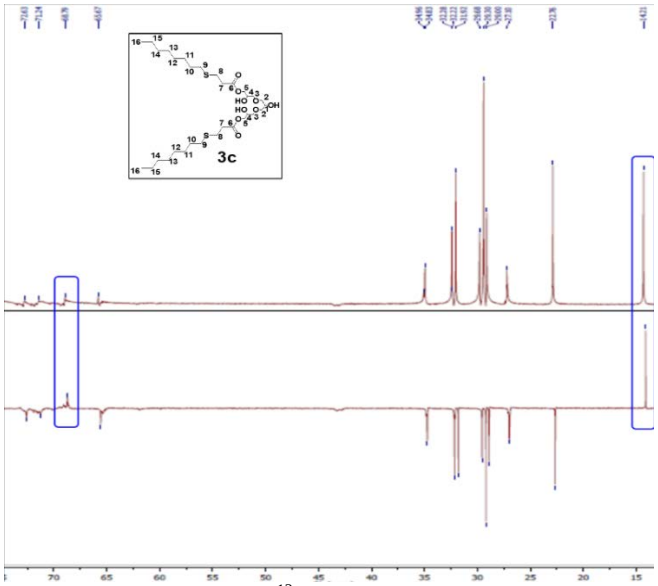


Figure 1. Proton decoupled ^{13}C NMR spectrum (above) and DEPT-135 carbon NMR spectrum (below) of compound **3c**.

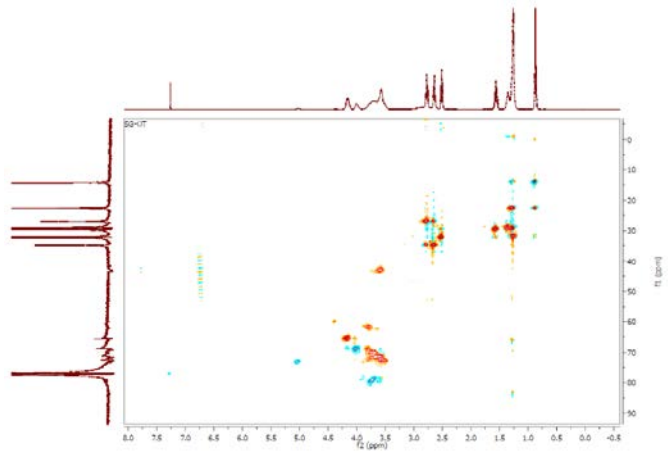


Figure 2. HSQC spectrum of compound **3c**

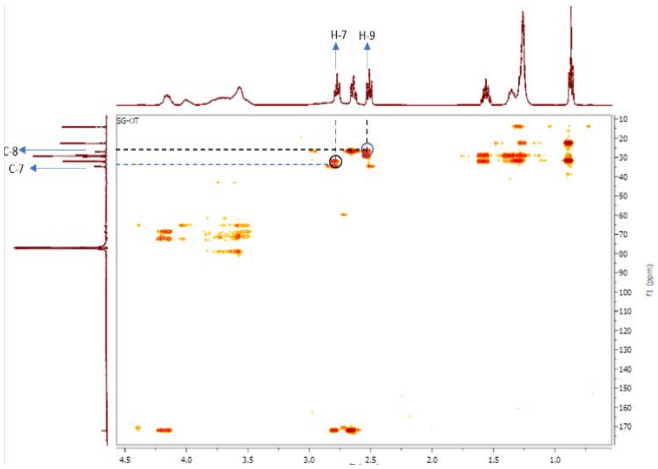


Figure 3. HMBC spectrum of compound **3c**

EXPERIMENTAL SECTION

Materials and Method

A new series of generation one [G-1] dendritic compounds were synthesized by varying the chain length of the

hydrophobic tail. All the reactions were performed under the argon atmosphere. All the solvents and reagents used in the synthesis were purchased from Merck/Sigma-Aldrich and used without further purification. TLC analysis was carried out under ultraviolet light for determining the progress of the reaction. ^{1}H NMR and ^{13}C NMR spectra were recorded on Bruker Avance 400 NMR spectrometer. Chemical shifts values are on δ scale and the coupling constant *J* values are in Hz. The IR spectras were recorded by a Alpha Bruker Model for liquid samples. The protocol for synthesis of compounds prepared and analytical data is as:

Synthesis of [G-1]-β-thiopropionate diesters

Aliphatic and aromatic [G-1]-β-thiopropionate diester were prepared by mixing [G-1] diacrylate (**1,** 1.0 g, 2.87 mmol), DMAP (0.73 g, 5.97 mmol) and pentathiol (**2a,** 1.49 g, 14.29 mmol)/ 3-methyl butane thiol (**2b,** 1.49 g, 14.29 mmol)/ octane thiol (**2c,** 2.09 g, 14.28 mmol)/ thio phenol (**4a,** 1.58 g, 14.33 mmol)/ benzyl mercaptum (**4b,** 1.78 g, 14.33 mmol) in a 100 ml dry round bottom flask under argon atmosphere. The reactions were then stirred for 24 h at room temperature and the progress was monitored using TLC (1:20 methanol:chloroform) until completion. Crude mixture was washed with hexane and the residue was dissolved in chloroform and washed three times with water. After that the organic layer shows no DMAP or Thiol, the was dried over Na_2SO_4 and the solvent was removed under vacuum to obtain the products (**3a-c, 5a-b**).

((2-hydroxypropane-1,3-diyl) bis(oxy)) bis (2-hydroxy propane-3,1-diyl) bis (3-(pentylthio) propanoate) (3a)

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 3.58-3.74 (m, 18H, 4H-5, 2H-4, 4H-3, 4H-2, 1H-1, 3C*H*-OH) 2.79 (t, 4H, *J*=8 Hz, H-8), 2.65 (t, 4H, *J*=8 Hz, H-7), 2.53 (t, 4H, *J*=8 Hz, H-9), 1.58 (tt, 4H, H-10), 1.31-1.37 (m, 8H, H-12 and H-11) and δ 0.90 (t, 6H, *J*=8 Hz, H-13).
^{13}C NMR ($CDCl_3$, 75 MHz) :δ 172.12 (C-6), 71.36 (C-3 and C-2), 68.79 (C-1 and C-4), 65.65 (C-5), 34.84 (C-7), 32.24 (C-9), 31.14 (C-10), 29.35 (C-11), 27.10 (C-8), 22.40 (C-12) and δ 14.09 (C-13).
IR (KBr) ν_{max}: 3459, 2924, 2867, 1736, 1457, 1427, 1347, 1300, 1244, 1177, 1133, 1054, 1033, 1014 and 720 cm^{-1}

((2-hydroxypropane-1,3-diyl) bis(oxy)) bis (2-hydroxy propane-3,1-diyl) bis (3-(isopentylthio) propanoate) (3b)

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 3.59-4.19 (m, 18H, 4H-5, 2H-4, 4H-3, 4H-2, 1H-1, 3C*H*-OH), 2.79 (t, 4H, *J*=8 Hz, H-8), 2.65 (t, 4H, *J*=8 Hz, H-7), 2.53 (t, 4H, *J*=8 Hz, H-9), 1.61-1.71 (m, 2H, H-11), 1.44-1.50 (m, 4H, H-10) and δ 0.90 (d, 12H, *J*=8 Hz, H-12 and H-13).
^{13}C NMR ($CDCl_3$, 75 MHz) :δ 172.05 (C-6), 71.23 (C-3 and C-2), 68.67 (C-1 and C-4), 65.58 (C-5), 38.53 (C-10), 34.75 (C-7), 30.11 (C-9), 27.46 (C-8), 26.99 (C-11), and δ 22.32 (C-12 and C-13).
IR (KBr) ν_{max}: 3423, 2955, 2155, 1737, 1054 and 720 cm^{-1}

((2-hydroxypropane-1,3-diyl) bis(oxy)) bis(2-hydroxy propane-3,1-diyl) bis(3-(octylthio) propanoate) (3c)

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 3.63-4.00 (m, 18H, 4H-5, 2H-4, 4H-3, 4H-2, 1H-1, 3C*H*-OH), 2.70 (t, 4H, *J*=8 Hz, H-8), 2.58 (t, 4H, *J*=8 Hz, H-7), 2.45 (t, 4H, *J*=8 Hz, H-9), 1.50 (qt, 4H, H-15), 1.20-1.23 (m, 20H, H-14, H-13, H-12, H-11 and H-10) and δ 0.81 (t, 6H, *J*=8 Hz, H-16).
^{13}C NMR ($CDCl_3$, 75 MHz) : δ 172.13 (C-6), 71.24 (C-3 and C-2), 68.79 (C-1 and C-4), 65.67 (C-5), 34.96 (C-7), 34.83 (C-9), 32.22 (C-10), 31.92 (C-13), 29.30 (C-11 and C-12), 29.00 (C-8), 27.10 (C-14), 22.76 (C-15) and δ 14.21 (C-16).
IR (KBr) ν_{max}: 3432, 2924, 2854, 1733, 1459, 1377, 1345, 1244, 1175, 1129, 937, 873, 735 and 703 cm^{-1}

((2-hydroxypropane-1,3-diyl) bis(oxy)) bis(2-hydroxy propane-3,1-diyl) bis(3-(phenylthio) propanoate) (5a)

^{1}H NMR ($CDCl_3$, 400 MHz) : δ 7.35 (d, 4H, H-10 and H-14), 7.27 (dd, 4H, H-11 and H-13), 7.20 (t, 2H, H-12), 3.46-4.15 (m, 18H, 4H-5, 2H-4, 4H-3, 4H-2, 1H-1, 3C*H*-OH), 3.16 (t, 4H, *J*=8 Hz, H-8) and δ 2.66 (t, 4H, *J*=8 Hz, H-7).
^{13}C NMR ($CDCl_3$, 75 MHz) : δ 171.84 (C-6), 135.15 (C-9), 130.25 (C-11 and C-13), 129.17 (C-10 and C-14), 126.75 (C-12), 71.31 (C-3 and C-2), 68.71 (C-1 and C-4), 65.47 (C-5), 34.34 (C-7) and δ 29.15 (C-8).
IR (KBr) ν_{max}: 3436, 2920, 2873, 2361, 2338, 1730, 1582, 1479, 1438, 1349, 1242, 1178, 1087, 1023, 738 and 691 cm^{-1}

((2-hydroxypropane-1,3-diyl) bis (oxy)) bis(2-hydroxy propane-3,1-diyl) bis (3-(benzylthio) propanoate) (5b)

^{1}H NMR ($CDCl_3$, 400 MHz) :δ 7.31 (d, 8H, H-11, H-12, H-14 and H-15), 7.30 (t, 2H, H-13), 3.56-4.16 (m, 18H, 4H-5, 2H-4, 4H-3, 4H-2, 1H-1, 3C*H*-OH), 3.48 (t, 4H, H-8), 2.69 (t, 4H, *J*=8 Hz, H-7) and δ 2.59 (t, 4H, *J*=8 Hz, H-9).
^{13}C NMR ($CDCl_3$, 75 MHz) : δ 172.99 (C-6), 138.09 (C-10), 128.98 (C-11 and C-15), 128.72 (C-12 and C-14), 127.28 (C-13), 71.37 (C-3 and C-2), 68.79 (C-1 and C-4), 65.46 (C-5), 36.40 (C-9), 34.44 (C-7) and δ 26.37 (C-8).
IR (KBr) ν_{max}: 3423, 2918, 2875, 2361, 2339, 1730, 1493, 1452, 1422, 1349, 1240, 1178, 1125, 1070, 768, 733 and 700 cm^{-1}

CONCLUSION

The single-step and economic method have been developed for the efficient synthesis of a novel series of five [G-1]-β-thiopropionate diesters compounds. These compounds with an acid-labile β-thiopropionate linker have been synthesized by Michael addition reaction between hydrophobic thiol and hydrophilic tri-glycerol diacrylate. The solvent-free reaction and purification by simply solvent extraction instead of any sophisticated chromatographic techniques have been the highlight of the design. All the synthesized compounds have been well characterized by using modern spectroscopic techniques like FT-IR, ^{1}H and ^{13}C NMR. Modern experimental techniques including DEPT, HMQC and HMBC have been used to better understand the spectral properties of these compounds. The ease of synthesis and purification of synthesized

compounds have opened up exciting possibilities of easy access to pH-responsive material for various therapeutic and biomedical applications.[21-24]

SUPPLEMENTARY INFORMATION

Experimental procedure and compound characterisation data has been provided as supporting information (available for download from article journal site).

Conflict of interest: Authors declare no conflict of interest.

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