

Lipase Immobilized on Magnetic Nanoparticles: A New Tool for Synthesis of Disulphide Compounds

Vennapusa Haritha, Harshadas Mitaram Meshram, Adari Bhaskar Rao*

Medicinal Chemistry & Pharmacology, CSIR-Indian Institute of Chemical Technology, Hyderabad, India
Email: *adarirao2002@yahoo.co.in

Received 19 January 2015; accepted 8 February 2015; published 11 February 2015

Copyright © 2015 by authors and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY).
<http://creativecommons.org/licenses/by/4.0/>



Open Access

Abstract

The study describes chemo-enzymatic synthesis of organic disulphide compounds. The reaction was initiated by hydrolysis of thiol acetates using hydrolytic enzyme lipase (PPL) immobilized on to magnetic nanoparticles and subsequent formation of organic disulphide compounds. Lipase was immobilized on to the magnetic nanoparticles by co-precipitation method via epichlorohydrin chitosan cross-linking, under mild and eco-friendly conditions. The immobilized lipase enzyme exhibited broad range of substrate specificity in synthesizing disulphide compounds, which involves both intra and inter-molecular disulphide bond formation under anaerobic conditions. The disulphide compounds synthesized also show a promising antimicrobial activity.

Keywords

Magnetic Nanoparticles, Lipases, Oxidative Coupling, Disulphide Compounds, Antimicrobial

1. Introduction

The disulphide linkages being an integral part of the macromolecules, mainly present in functional proteins like enzymes, peptides, hormones and antibodies. These disulphide bonds provide configurational specificity and also help in stabilizing the protein molecules to carry out their specific biological functions [1]-[7]. The synthesis of organic disulphide compounds by the oxidation of thiol derivatives has become a common practice, and quite a few methodologies have been developed in the preparation of these compounds [8] [9]. There has been an increasing demand for novel, ecofriendly and efficient methodologies for the synthesis of organic disulphide compounds owing to their importance in a wide range of pharmaceutical and agro-chemical applications [10] [11].

*Corresponding author.

The instability of the thiol derivatives, due to air oxidation, has led to masking/protecting the thiol groups, which is an important process in multistep synthesis of bioactive molecules. So, there is a continuous research interest for the cleavage of thiol derivatives and subsequent formation of disulphide compounds. Transition metal-catalyzed reactions have made a great contribution to the preparation of disulphide compounds; however most of these methods have long reaction time, tedious workup and use of expensive reagents [12] [13]. These limitations necessitate in developing novel and efficient methods for the synthesis of disulphide compounds in mild and environmental friendly conditions. In continuation of our work to explore new methodologies in the area of synthesis of biological active compounds using biocatalysts [14] [15], we herein report the synthesis of organic disulphide compounds by the oxidative cleavage of thiol acetates using inexpensive immobilized lipases (**Scheme 1**).

2. Experimental

2.1. Materials

All the chemicals used in the study were AR grade and were purchased from Merck co and Sigma-Aldrich Chemicals USA. Lipases obtained from porcine pancreas (PPL) 3000 U/mg of protein (Biuret) EC 232-619-9 from Sigma—Aldrich USA. All the microbial strains used were procured from ATCC microbial culture collection center—USA.

2.2. HPLC

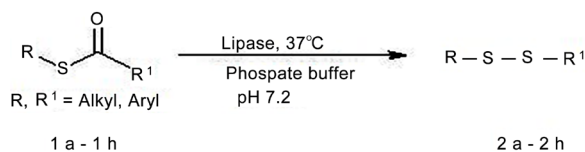
The disulphide products were quantified by HPLC (Gilson system) using silica column (inside diam. 4.6×250 mm, film thickness $4 \mu\text{m}$); with a mobile phase of Hexane: Ethyl acetate (70:30); flow rate 0.8 ml/min at a UV wave length of 254 nm.

2.3. Preparation of Lipase-Immobilized Magnetic Nanoparticles

The magnetic nanoparticles (Magnetite (Fe_3O_4)-chitosan (CS)-poly[N-benzyl-2-(methacryloxy)-N,N-dimethylethanaminium bromide] (PQ) nanoparticles (**Fe_3O_4 -CS-PQ**) were synthesized as reported in the literature [16]. The immobilization of lipase on to the surface of chitosan coated magnetic nanoparticles was performed by the formation of an amide bond between the carboxyl group of lipase and the primary amino group of the nanoparticles. Lipase (0.1 g) was dispersed in 0.1 M sodium phosphate buffer pH 8.0 (10 mL) at 20°C and incubated for 2 hours in shaking incubator. The magnetic nanoparticles (1.0 g) were added to the above lipase enzyme solution and further incubated at 180 rpm for 4 hours. The immobilized enzyme bound to the nanoparticles was separated from the reaction mixture using an external magnet and lyophilized to obtain white powder [17]. It was observed that 15% of protein was bound to the immobilized matrix; the specific activity of the enzyme lipase was found to be 35 Units/mg of protein. The size of nanoparticles and immobilization of lipase onto magnetic nanoparticles was characterized using TEM images. As observed from the images the size of the particles were in the range of 6 to 26 nm with an average particle size of 13 - 17 nm (**Figure 1**).

2.4. Assay of Lipase Activity

The lipase activity for free and immobilized enzyme was measured by the hydrolysis of olive oil esters as described [18] [19]. The olive oil ester (10 mL) and gum arabic solution (5 mL, 7%, w/v) was mixed with free (0.1 ml, 6.47 mg/mL) or immobilized lipase (5 mg nanoparticles) dispersed in 2 ml of 0.1 M phosphate buffer pH 7.5. The hydrolysis of fatty acid ester was carried out at 45°C for 15 min and the reaction was stopped by adding 1mL of ethanol-acetone solution (1:1). The liberated fatty acid in the medium was titrimetrically determined using 0.01 M NaOH solution using phenolphthalein indicator. From the titrimetric analysis, the immobilized lipase enzyme activity was found to be 150 U/mg protein.



Scheme 1. Enzymatic synthesis of disulphide compounds.

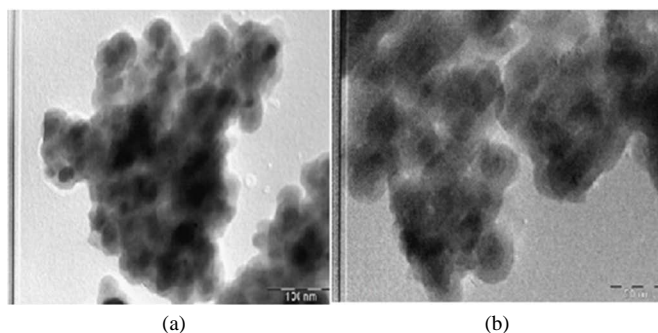


Figure 1. TEM: (a) Amine functionalized silica coated magnetic nanoparticles; (b) Lipase enzyme immobilized MNPs.

2.5. Lipase Assisted Synthesis of Organic Disulphide Compounds

The enzymatic hydrolysis of thiophenyl compounds *i.e.*, the substituted aromatic, heterocyclic and aliphatic thio esters (**1a-1h**) (1m mol) were taken in 10 ml of solvent cyclohexane, to the above reaction medium the dispersed lipase enzyme immobilized on to the magnetic nanoparticles was added and the reaction was incubated under nitrogen atmosphere on shaking incubator at 35°C. The rate of product formation was monitored by TLC/HPLC. On completion of reaction (~55% conversion), the lipase (PPL) immobilized on magnetic nanoparticles present in the reaction mixture was separated with the help of an external magnetic bar, and the immobilized enzyme thus obtained was reused. The crude disulphide product obtained was purified by flash chromatography and the structures of the products were confirmed by NMR, Mass spectral studies.

2.6. Antimicrobial Assay

The antimicrobial and antifungal activities of the disulphide compounds were determined using the dilution technique. Minimum inhibitory concentrations (MICs) were determined [20] against two gram positive bacteria *Staphylococcus aureus* (ATCC 29737) and *Bacillus cereus* (ATCC 14603) and three gram negative bacteria *Escherichia coli* (ATCC 10536), *Klebsiella pneumoniae* (ATCC 13883) and *Pseudomonas aeruginosa* (ATCC 25619), as well as against the pathogenic fungi *Candida albicans* (ATCC 53324), and *C. tropicalis* (ATCC 13801). Standard antibiotics netilmicin and amphotericin B were used as positive controls for screening antimicrobial and antifungal activities.

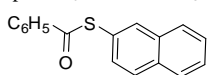
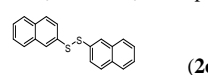
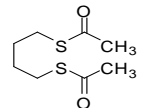
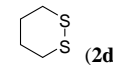
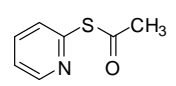
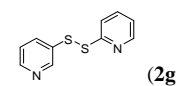
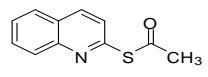
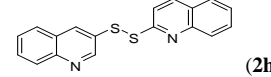
3. Results and Discussion

The hydrolytic enzyme lipase immobilized on magnetic nano particles, has shown broad substrate specificity in hydrolyzing both aromatic & aliphatic thiol acetates and simultaneously forms high yield of the respective disulphide compounds (**Table 1**). On repetitive use of immobilized enzymes (5 cycles) the product (**2a-2h**) formation was reduced by 8% - 15% when compared to initial yields. The results show the short chain esters *i.e.*, two carbon chains cleaved smoothly, whereas in case of longer chain acetates the reaction was very sluggish. In the processes of hydrolytic reaction no intermediate compound was observed, so it is assumed that the enzymatic hydrolysis of organic thioesters generate thiol radical, resulting in spontaneous formation of disulphide compounds in anaerobic conditions [21]. It was interesting to note that thioesters (**2d**) underwent smooth cleavage in the mild and environmental friendly conditions resulting in high yields of cyclic disulphide compounds. The biocatalytic method developed may be an attractive alternative to the existing methodologies in the synthesis of aliphatic, aromatic and hetroaromatic disulphide compounds (**2a-2h**) [22]-[24]. The synthesized disulphide compounds (**2a-2h**) exhibited promising antimicrobial activity against different bacterial and fungal strains. The MICs of the synthetic disulphide compounds against the pathogenic microorganisms were presented in **Table 2**. From the data it was observed that the compound (**2b**) has shown potent anti microbial activity when compared to corresponding thiazoles. Thus the study confirms a novel methodology in synthesis of organic disulphide compounds in an environment friendly condition.

4. Conclusion

In conclusion, we have demonstrated the lipase assisted oxidative coupling of thiol acetate into disulphide

Table 1. Lipase immobilized magnetic nanoparticles mediated synthesis of disulphide compounds.

S. No	Substrate	Product	Yield %
1	C ₆ H ₅ -SCOCH ₃	C ₆ H ₅ -S-S-C ₆ H ₅ (2a)	42
2	C ₆ H ₅ -S-CO-t-Bu	2a	36
3	C ₆ H ₅ -S-CO-C ₂ H ₅	2a	48
4	C ₆ H ₅ -S-CO-C ₂ H ₅	2a	40
5	C ₆ H ₅ -S-CO-C ₁₅ H ₃₁	2a	52
6	C ₆ H ₅ -S-CO-C ₆ H ₅	2a	36
7	C ₆ H ₅ -S-CO-C ₆ H ₄ -p-Cl	2a	47
8	p-C ₆ H ₅ -S-CO-C ₆ H ₄ -CO-S-C ₆ H ₅	2a	50
9	p-Cl-C ₆ H ₄ -S-CO-CH ₃	p-Cl-C ₆ H ₄ -S-S-C ₆ H ₄ -Cl-p (2b)	55
10		 (2c)	45
11		 (2d)	38
12	n-C ₃ H ₇ -S-CO-CH ₃	n-C ₃ H ₇ -S-S-C ₃ H ₇ -n (2e)	40
13	n-C ₁₂ H ₂₅ -S-CO-CH ₃	(n-C ₁₂ H ₂₅ -S) ₂ (2f)	43
14	n-C ₁₂ H ₂₅ -S-CO-t-Bu	2f	39
15	n-C ₁₂ H ₂₅ -S-CO-C ₃ H ₅	2f	30
16		 (2g)	38
17		 (2h)	33

^aAll the compounds were characterized by the ¹HNMR, MS, IR Spectral studies. ^bYeilds refer to pure after column chromatography.

Table 2. Antimicrobial activities^a (MIC in µg·ml⁻¹).

Compound	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>B. subtilis</i>	<i>S. aureus</i>	<i>C. albicans</i>	<i>C. tropicalis</i>
2a	-	-	-	-	-	>2.5	>2.5
2b	15.0	20.0	10.0	10.5	30.5	>2.5	>2.5
2c	20.5	25.0	35.0	20.0	50.0	>2.5	>2.5
2d	10.0	50.0	50.0	25.0	70.0	>2.5	>2.5
2e	35.0	35.0	30.0	15.0	20.0	>2.5	>2.5
2f	12.5	20.5	25.0	60.0	50.0	>5.0	>5.0
Amphot-ericin B	-	-	-	-	-	3.00	3.00
Netilmicin	1.00	1.00	1.00	1.00	1.00	-	-

^aResults obtained were the mean of three independent experiments, with standard deviation of 10%.

compounds using immobilized lipase on to magnetic nanoparticles. Thus the methodology developed and carried out in mild and environment friendly condition. Further, we believe that the application of this methodology can be used in the synthesis of biologically important compounds having disulphide linkage and this methodology is an attractive alternative to the existing method.

Acknowledgements

Authors are thankful to CSIR-IICT XII FYP Project CSC-0106 "BIAGOS" for funding the research project.

References

- [1] James, P.T., Cui, R.W., Wen, L and Jing, W.Z. (1991) Disulfide Bond Formation in Peptides by Dimethyl Sulfoxide. Scope and Applications. *Journal of the American Chemical Society*, **113**, 6657-6662. <http://dx.doi.org/10.1021/ja00017a044>
- [2] Zhengkai, L., Fang, K., Hang, D., Hualong, X., Haifeng, X and Xiangge, Z. (2013) Synthesis of Disulfides and Diselenides by Copper-Catalyzed Coupling Reactions in Water. *Organic & Biomolecular Chemistry*, **11**, 2943-2946. <http://pubs.rsc.org/en/content/articlelanding/2013/ob/c3ob40464a>
<http://dx.doi.org/10.1039/c3ob40464a>
- [3] Rudolf, M., Stephan, K., Thomas, L., Hanno, L., Thomas, E. and Bernd, G. (1984) Applications of Synthetic Peptides. *Angewandte Chemie*, **24**, 719-727. <http://onlinelibrary.wiley.com/doi/10.1002/anie.198507193/pdf>
- [4] Carolyn, S.S. and Chris A.K. (2002) Formation and Transfer of Disulphide Bonds in Living Cells. *Nature Reviews Molecular Cell Biology*, **3**, 836-847. <http://www.nature.com/nrm/journal/v3/n11/abs/nrm954.html>
<http://dx.doi.org/10.1038/nrm954>
- [5] Leena, K., Pankaj, K., Chandramukhi, S.P. and Siva, S.P. (2013) Synthesis of Various S-S Linked Symmetric Bisazaheterocycles: A Review. *Mini-Reviews in Organic Chemistry*, **10**, 268-280. <http://benthamscience.com/journal/abstracts.php?journalID=mroc&articleID=113519>
<http://dx.doi.org/10.2174/1570193X11310030006>
- [6] Wilkes, B.C., Hruby, V.J., Castrucci, A.M., Sherbrooke, W.C. and Hadley, M.E. (1984) Synthesis of a Cyclic Melanotropic Peptide Exhibiting both Melanin-Concentrating and -Dispersing Activities. *Science*, **224**, 1111-1113. <http://www.sciencemag.org/content/224/4653/1111.abstract>
- [7] Hiram, F. G. (1997) Protein Disulfide Isomerase and Assisted Protein Folding. *The Journal of Biological Chemistry*, **272**, 29399-29402. <http://www.jbc.org/content/272/47/29399>
<http://dx.doi.org/10.1074/jbc.272.47.29399>
- [8] Harshadas, M. (1993) An Efficient and Mild Cleavage of Thiol Acetate with Clayfen in the Absence of Solvent. *Tetrahedron Letters*, **34**, 2521-2522. <http://www.sciencedirect.com/science/article/pii/S0040403900604574>
[http://dx.doi.org/10.1016/S0040-4039\(00\)60457-4](http://dx.doi.org/10.1016/S0040-4039(00)60457-4)
- [9] Szajewski, R.P and Whitesides, G.M. (1980) Rate Constants and Equilibrium Constants for Thiol-Disulfide Interchange Reactions Involving Oxidized Gluthathione. *Journal of the American Chemical Society*, **102**, 2011-2026. <http://dx.doi.org/10.1021/ja00526a042>
- [10] Ayodele, E.T., Olajire, A.A., Amuda, O.S. and Oladoye, S.O. (2003) Synthesis and Fungicidal Activity of Acetyl Substituted Benzyl Disulfides. *Bulletin of the Chemical Society of Ethiopia*, **17**, 53-60. <http://www.readcube.com/articles/10.4314/bcse.v17i1.61731>
- [11] Field, L. and Oae, S. (Ed.) (1977) *Organic Chemistry of Sulfur*. Plenum, London, 205.
- [12] Sato, T., Otera, J. and Nozaki, H. (1990) Activation and Synthetic Applications of Thiostannanes. Efficient Conversion of Thiol Acetates into Disulfides. *Tetrahedron Letters*, **31**, 3595-3596. <http://www.sciencedirect.com/science/article/pii/S0040403900944514>
[http://dx.doi.org/10.1016/S0040-4039\(00\)94451-4](http://dx.doi.org/10.1016/S0040-4039(00)94451-4)
- [13] Anthony, P.B., John, A.M., Christopher, W.P and Nicholas, F.W. (1993) Radical-Induced Fragmentations of Ketoperoxides. *Tetrahedron*, **49**, 10643-10654. <http://www.sciencedirect.com/science/article/pii/S0040402001815544>
[http://dx.doi.org/10.1016/S0040-4020\(01\)81554-4](http://dx.doi.org/10.1016/S0040-4020(01)81554-4)
- [14] Bhaskar, R.A., Rehman, H., Krishnakumari, B. and Yadav, J.S. (1994) Lipase Catalysed Kinetic Resolution of Racemic (\pm) 2,2-dimethyl-3-(2-methyl-1-propenyl)-cyclopropane Carboxyl Esters. *Tetrahedron Letters*, **35**, 2611-2614. <http://www.sciencedirect.com/science/article/pii/S0040403900771863>
[http://dx.doi.org/10.1016/S0040-4039\(00\)77186-3](http://dx.doi.org/10.1016/S0040-4039(00)77186-3)
- [15] Yadav, J.S., Bhaskar, R.A., Ravindra, R.Y. and Venkata, R.R.K. (1997) Enzymatic Resolution of (\pm)-Cis-3-(2,2-dichloro-3,3,3-trifluoropropyl)-2,2-dimethylcyclopropane Carboxylate. *Tetrahedron: Asymmetry*, **8**, 2291-2294. <http://www.sciencedirect.com/science/article/pii/S0957416697002516>
[http://dx.doi.org/10.1016/S0957-4166\(97\)00251-6](http://dx.doi.org/10.1016/S0957-4166(97)00251-6)
- [16] Tomasz, S., Marta, Z.B and Michal, P.M. (2013) Lipase-Immobilized Magnetic Chitosan Nanoparticles for Kinetic Resolution of (R,S)-Ibuprofen. *Journal of Molecular Catalysis B: Enzymatic*, **94**, 7-14. <http://www.sciencedirect.com/science/article/pii/S138117713001070>

- <http://dx.doi.org/10.1016/j.molcatb.2013.04.008>
- [17] Xun, E.-N., Lv, X.-L., Kang, W., Wang, J.-X., Zhang, H., Wang, L. and Wang, Z. (2012) Immobilization of *Pseudomonas fluorescens* Lipase onto Magnetic Nanoparticles for Resolution of 2-Octanol. *Applied Biochemistry and Biotechnology*, **168**, 697-707. <http://link.springer.com/article/10.1007%2Fs12010-012-9810-9>
<http://dx.doi.org/10.1007/s12010-012-9810-9>
- [18] Bayramoglu, G. and Arica, M.Y. (2008) Preparation of Poly(glycidylmethacrylate-methylmethacrylate) Magnetic Beads: Application in Lipase Immobilization. *Journal of Molecular Catalysis B: Enzymatic*, **55**, 76-83.
<http://www.sciencedirect.com/science/article/pii/S1381117708000544>
<http://dx.doi.org/10.1016/j.molcatb.2008.01.012>
- [19] Zhang, D.H., Yuwen, L.X., Xie, Y.L., Wei, L. and Li, X.B. (2012) Improving Immobilization of Lipase onto Magnetic Microspheres with Moderate Hydrophobicity/Hydrophilicity. *Colloids and Surfaces B: Biointerfaces*, **89**, 73-78.
<http://www.sciencedirect.com/science/article/pii/S0927776511005194>
<http://dx.doi.org/10.1016/j.colsurfb.2011.08.031>
- [20] Laila, H.A., Rafat, M.E., Lobna, A.E.N., Ahmed, M.A., Mohamed, I. and Amin, A.S. (2014) Metal Based Pharmacologically Active Agents: Synthesis, Structural Characterization, Molecular Modeling, CT-DNA Binding Studies and *in Vitro* Antimicrobial Screening of Iron(II) Bromosalicylidene Amino Acid Chelates. *Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy*, **117**, 366-378.
<http://www.sciencedirect.com/science/article/pii/S1386142513008044>
<http://dx.doi.org/10.1016/j.saa.2013.07.056>
- [21] Carl, R.J. (1998) Biotransformations in the Synthesis of Enantiopure Bioactive Molecules. *Accounts of Chemical Research*, **31**, 333-341. <http://dx.doi.org/10.1021/ar970013q>
- [22] Barry, M.T. (1978) *a*-Sulfonylated Carbonyl Compounds in Organic Synthesis. *Chemical Reviews*, **78**, 363-382.
<http://dx.doi.org/10.1021/cr60314a002>
- [23] Alban, C., Rahul, A.W., Srijit, B., Andreas, O., Per, J.R.S. and Joseph, S.M.S. (2014) One-Pot Synthesis of Keto Thioethers by Palladium/Gold-Catalyzed Click and Pinacol Reactions. *Organic Letters*, **16**, 5556-5559.
<http://dx.doi.org/10.1021/ol502553p>
- [24] Scott, E.D., Sergio, R., Matthew, P.W. and Hao, W. (2014) Catalytic, Enantioselective Sulfonylation of Ketone-Derived Enoxysilanes. *Journal of the American Chemical Society*, **136**, 13016-13028.
<http://dx.doi.org/10.1021/ja506133z>

Spectroscopic Data

Phenyl disulphide (2a): White solid; mp: 57°C - 59°C [mp: 58°C - 60°C]. ¹H NMR (300 MHz, CDCl₃): δ = 7.40 - 7.35 (m, 4 H), 7.21 - 7.10 (m, 6 H). ¹³C NMR (300 MHz, CDCl₃): δ = 137.02, 129.05, 127.45, 127.11. MS: *m/z* = 219 (M + H).

p-Chlorophenyl disulphide (2b): White solid; mp: 72°C - 74°C [mp: 72°C - 73°C]. ¹H NMR (300 MHz, CDCl₃): δ = 7.33 (d, J = 8.8 Hz, 4 H), 7.18 (d, J = 8.8 Hz, 4 H). ¹³C NMR (300 MHz, CDCl₃): δ = 135.12, 133.66, 129.33, 129.32. MS: *m/z* = 286 (M⁺, 64)

2-Naphthhenyl disulphide (2c): White solid; mp: 136°C - 137°C [mp: 135°C - 138°C]. ¹H NMR (300 MHz, CDCl₃): δ 7.98 (d, J = 0.9 Hz, 2 H), 7.80 - 7.22 (m, 4 H), 7.75 - 7.61 (m, 2 H), 7.47 - 7.42 (m, 4 H). ¹³C NMR (300 MHz, CDCl₃): δ = 134.00, 133.52, 132.50, 128.89, 127.80, 127.51, 126.70, 126.66, 126.20, 125.74. MS: *m/z* = 318 (M⁺)

1, 2-dithiane (2d): White solid. m.p: 30°C - 32°C. ¹H NMR (300 MHz, CDCl₃): 1.6 - 1.7 (m, 4 H), 2.5 - 2.8 (4 H, m). ¹³C NMR (300 MHz, CDCl₃): δ 26.70, 37.45. MS: *m/z* = 120 (M⁺, 61)

n-Propyl disulphide (2e): Colourless oil. ¹H NMR (300 MHz, CDCl₃): δ = 2.61 (t, J = 7.1 Hz, 4 H), 1.65 (sp, J = 7.5 Hz, 4 H), 0.99 (t, J = 7.3 Hz, 6 H). ¹³C NMR (300 MHz, CDCl₃): δ 40.90, 22.34, 12.90. MS: *m/z* = 150 (M⁺, 90)

Dodecyl disulphide (2f): Colourless oil. ¹H NMR (300 MHz, CDCl₃): δ = 2.48 (t, J = 6.9 Hz, 4 H), 1.64 - 1.54 (m, 4 H), 1.35 - 1.27 (m, 36 H), 0.89 (t, J = 6.3 Hz, 6 H). ¹³C NMR (300 MHz, CDCl₃): δ 34.50, 31.90, 29.60, 29.60, 29.50, 29.50, 29.30, 29.10, 28.42, 24.60, 22.61, 14.00. MS: *m/z* = 402 (M⁺).

Scientific Research Publishing (SCIRP) is one of the largest Open Access journal publishers. It is currently publishing more than 200 open access, online, peer-reviewed journals covering a wide range of academic disciplines. SCIRP serves the worldwide academic communities and contributes to the progress and application of science with its publication.

Other selected journals from SCIRP are listed as below. Submit your manuscript to us via either submit@scirp.org or **Online Submission Portal**.

