DESIGN OF MULTIFUNCTIONAL MOLECULE FOR TREATMENT OF ACNE. SYNTHESIS OF SKIN SOLUBLE BENZOPHENONE PEROXYESTERS

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ABSTRACT

Dr. Douglas C. Neckers, Advisor

Acne vulgaris is the most common skin disorder whose pathogenesis appears to be multifactorial. A synthetic scheme for a new multifunctional peroxyester compound for acne treatment was proposed and successfully completed. This compound **(8)** contains three parts which are expected to serve different functions. A *tert-Bu* perester moiety would serve as radical generating part for acne treatment. The benzophenone chromophore would photochemically control radical generation. Finally, the diethyleneglycol methyl ether part is expected to increase the skin affinity of the proposed molecule.

Compound **(8)** shows characteristic absorption of the benzophenone chromophore with maxima at 348 and 256 nm.

Combinatorial experiments were performed by reacting eight different alcohols with methyl 4-(4-bromomethylbenzoyl) benzoate **(4)** as the common substrate.

To my grandfather, who did not live to see it

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CHAPTER 1. BACKGROUND AND INTRODUCTION

1.1 Pathogenesis of acne

Acne vulgaris is the most common dermatologic disease, affecting approximately 17 million people in the United States. More than 80% of the U.S. population develops some form of acne during their lifetime.[1a] In a study of 749 adults between the ages of 25-58, 54% of women and 40% of men had some form of acne.[1b] Lesions develop sooner in females than in males due to their earlier onset of puberty, usually occurring between ages 16-17 and 17-19 in females and males respectively.[3] Acne is not usually a problem for the elderly; however, there is a small number of individuals experience acne in their sixth and seventh decades. These persons may have suffered from it for 30-60 years or more.[3] This disease can have far-reaching and severe phychosocial effects in adolescents and young adults during a time of life which is burdened with difficult sexual and social issues and intense interpersonal relationships.[2] The pathogenesis of acne is multifactored, involving increased sebum production of the pilosebaceous units, follicular epithelial desquamation, bacterial proliferation, and inflammation. Acne lesions arise from pilosebaceous units, which consist of sebaceous glands and small hair follicles.

1.1.1 The pilosebaceous unit

The pilosebaceous unit is the location of development of acne (Figure 1)[3] and consists of sebaceous glands and hair follicles, which are connected to the surface of the skin by a duct called the infundibulum. These units are found everywhere on the body except the palms and soles and are in greatest density on the face, upper neck and chest.[8]

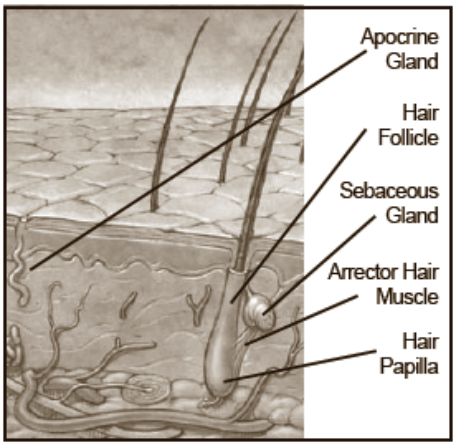


Figure 1. The pilosebaceous unit
(Reproduced from reference 3)

Pilosebaceous units atrophy shortly after birth, but become larger and active (increased sebum production) because of the influence of androgens, mainly dehydroepiandrosterone sulfate (DHEAS), at the onset of adolescence.[4] The end products of bacterial metabolism normally flow out of the pilosebaceous unit along with sebum.[5] Sebum is an excretory product of sebaceous glands made of lipids, keratin and bacteria in a complex mixture of triglycerides, fatty acids, wax esters and cholesterol.[6] Different hypotheses have been made and studied to explain its role. It is generally known that sebum provides lubrication to the skin, and helps to avoid water loss to maintain 10% hydration of skin and hair.[7a]

1.1.2 Stages of acne

However, excess sebum production combined with increased epithelial cell leads to the formation of microcomedones, a microscopic lesion not visible with the naked eye. The microcomedo becomes larger and visible by increasing addition of cells and sebum to the plug.[7b] It appears as a small, pale nodule and usually known as *closed* comedo (Figure 2C), often termed “whiteheads”. An *open* comedo, usually known as

"blackheads" (Figure 2B), appears when too much material accumulates behind the plug and the opening of the follicle becomes wider, permitting the plug to protrude outward.[3] The hair shaft in the follicle plays an important role. For example, thin and small hair can be encased in the plug; however thicker hair such as those of the scalp and beard push the developing plug outward and as a result prevent comedo formation.

The combination of sebum and desquamated cells presents an environment that is mature for the growth of *Propionibacterim acnes (P. acnes)*, the principal organism in inflammatory acne lesions. They colonize within pilosebaceous follicles but it is unknown whether they are capable of multiplication on the skin surface. Furthermore, it is unsure whether they are present in follicles permanently or temporarily and whether their presence should be considered as an infection or expression of commensalisms with other systems.[7c] *P. acnes* produces an extracellular lipase that hydrolyzes sebum triglycerides to glycerol and free fatty acids. The free fatty acids, which are produced are proinflammatory.[7d] Thus, the irritation causes stimulation of an immune response, which results in development of inflammatory lesions – papule and pustule (Figure 2D and 2E).[8]

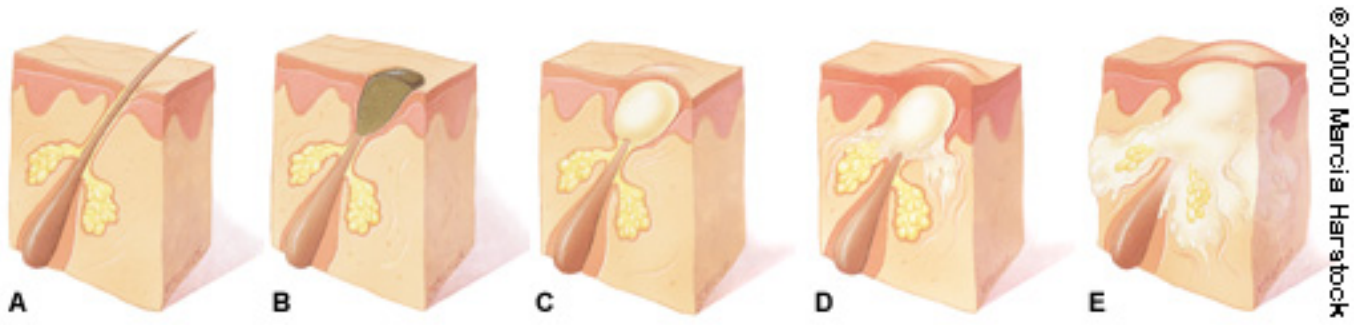


Figure 2. Stages of acne: *(A)* normal follicle; *(B)* open comedo (blackhead); *(C)* closed comedo (whitehead); *(D)* papule; *(E)* pustule. (Reproduced from reference 8)

1.2 Types of acne

Acne is often categorized according to the Pillsbury classification,[9] which categorizes acne into four grades according to the degree and severity of lesions. In grade I acne there is a predominance of noninflammatory lesions or comedones, both open and closed, but there may also be a few small inflammatory lesions: papules or pustules. Grade II acne consists of comedones, papules and pustules; however, the lesions are mainly limited on the face. Patients with grade III acne have involvement of the upper trunk and shoulders, with numerous papules and pustules and a few larger lesions. Grade IV acne consists mainly of large, painful cystic lesions which are usually on the face and upper trunk.

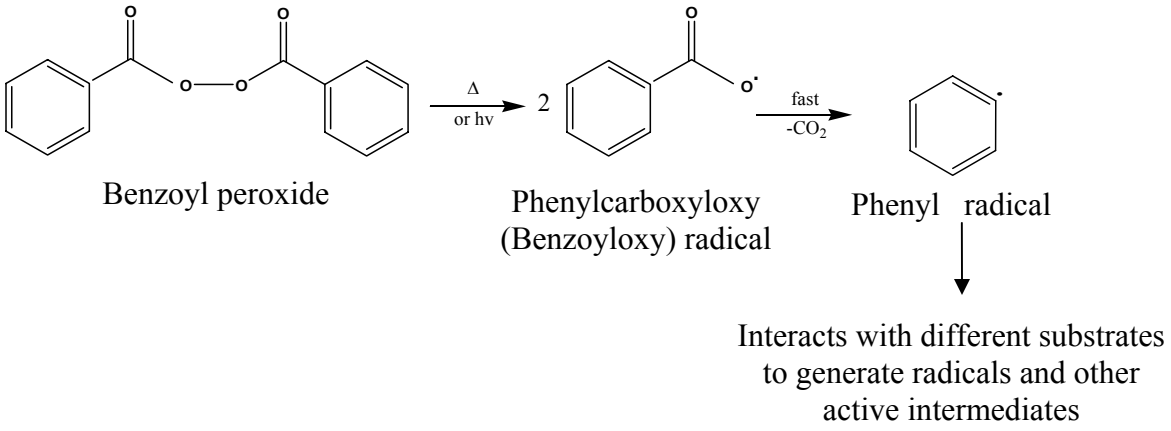
1.3 Topical agents for acne treatment

Topical treatments for acne include benzoyl peroxide (BP), retinoids, antibiotics, azelaic acid, and salicylic acid.[2]

1.3.1 Benzoyl peroxide

Benzoyl peroxide (BP) is the most frequently used topical agent for acne treatment. It is available both in prescription (2.5% to 10%) and nonprescription formulations.[10] BP is a powerful oxidizing agent that causes fast and prolonged reduction of free fatty acids and inhibits the growth of *P. acnes*. It also functions as a mild "comedolytic" agent by increasing epithelial cell turnover with desquamation. This agent comes in water-based or alcohol-based preparations. The water based formulations are less drying than the alcohol based preparations. Benzoyl peroxide gels are applied once or twice daily. The most common side effects of benzoyl peroxide consist mainly of skin irritation including burning, blistering, crusting, severe redness, and skin rash. The skin

irritation effect occurs more often at higher concentrations and is reported in 1 to 2 percent of patients.[11] The general decomposition scheme (Scheme 1) shown below illustrates the importance of the free radical formation as the main factor in biological activity of BP.[12]



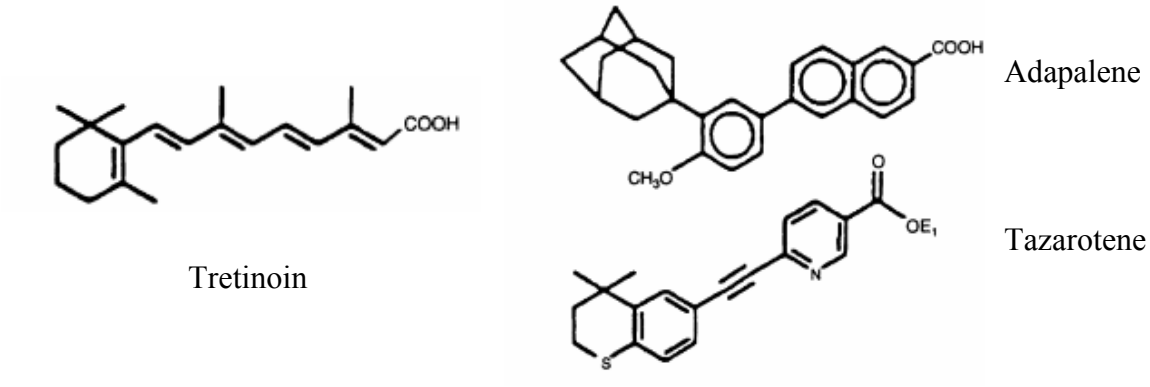
Scheme 1. Decomposition of benzoyl peroxide via a homolytic bond cleavage.

The exact mechanisms of action of BP and other peroxides in the acne treatment process are not well understood and further mechanistic studies are needed.

1.3.2 Topical retinoids

Retinoids are derivatives of Vitamin A, which function by slowing the desquamation process and decreasing the number of comedones. Retinoids are currently the most effective "comedolytic" agents for the treatment of acne. Retinoids, as an important therapeutic class, have a substantial effect on epithelia and have revolutionized dermatologic treatment in the last twenty years. First-generation retinoids include retinol (vitamin A) and its active metabolites, which include tretinoin and isotretinoin. Tretinoin

has been the main agent of topical retinoid therapy for some time. It is available as a cream, gel, or liquid. Second-generation retinoids include etretinate and acitretin. Extensive molecular modification has produced a third generation of polyaromatic retinoids known collectively as arotinoids (e.g., adapalene and tazarotene). Several studies with *in vitro* and *in vivo* human cell and animal models have shown adapalene to have moderate to strong anti-inflammatory activity.[13] The structures of tretinoin, adapalene and tazarotene, are shown in Figure 3.



Tretinoin

Adapalene

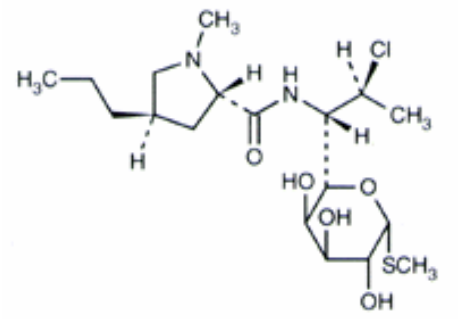
Tazarotene

Figure 3. Structures of retinoids used in acne treatment .

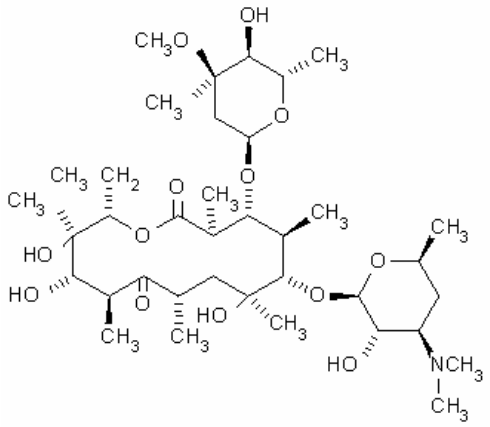
All tretinoin formulations can cause some skin irritation, with liquids being the most irritating and creams the least irritating. Irritation is less of a problem with adapalene. Studies using topical retinoids as monotherapy have shown not only significant reductions in comedones, but also a significant reduction in papulopustular lesions.[14]

1.3.3 Antibiotics

Antibiotics prescribed for acne include tetracyclines, erythromycin and clindamycin, all of which target *P. acnes.* Antibiotics, including clindamycin and erythromycin (Figure 4.), are available in different forms: solutions, lotions, gels, etc. These agents reduce the population of *P. acnes* in the pilosebaceous duct and have a mild "comedolytic" effect. They also demonstrate anti-inflammatory effects.[11] In clinical trials with topical antibiotics, a small (20%) but consistent reduction in the number of comedones was reported. This is in contrast to 60% reduction reported with tretinoin, adapalene and tazarotene.[14] It has also been shown that systemic antibiotics can be associated with gastrointestinal disturbance, vaginal candidiasis[15] and effects on the central nervous system.



Clindamycin



Erythromycin

Figure 4. Structures of clindamycin and erythromycin

1.3.4 Azelaic acid

Azelaic acid is a C-10 dicarboxylic acid that was first investigated in the 1970s as a treatment for hyperpigmentation and was coincidentally found to be an effective acne

treatment.[16] Although the exact mechanism of its action is unknown, this agent has antibacterial activity, and it appears to be as effective as benzoyl peroxide or tretinoin in the treatment of mild to moderate acne. Azelaic acid is available as a 20 percent cream (Azelex), which is applied twice daily to a clean, dry affected area. This agent is fairly well tolerated, with only about 5 percent of patients complaining of transient cutaneous irritation and erythema .[17]

Salicylic acid

Salicylic acid can help combat comedones by breaking down follicular plugs and by reducing the rate of follicular desquamation. Usually it is available at a concentration of 0.5 or 2 percent in a number of creams and lotions. It has been shown to be as effective as benzoyl peroxide in the treatment of comedonal acne.[18] However, it is also an irritant, causing erythema and peeling, and even exacerbations of inflammatory acne lesions.[19]

1.3.5 Treatment vehicle

In the acne treatment process the vehicle (cream, gel, lotion or solution) may be as important as the active acne treatment agent. The choice of vehicle depends on the nature of skin. For patients who have oily skin gels may be used, which have a drying effect. However, gels may cause a burning type irritation in some cases. Creams are suitable for patients with sensitive or dry skin. It requires nonirritating and nondrying formulations.

It has become clear that the choice of agents used to treat acne involves multiple factors such as the severity of lesion, duration of disease, past and present response to therapy, and tendency for scarring and pigmentation. All acne treatment reagents described above, covering all disease variants, are shown in Table 1. [14]

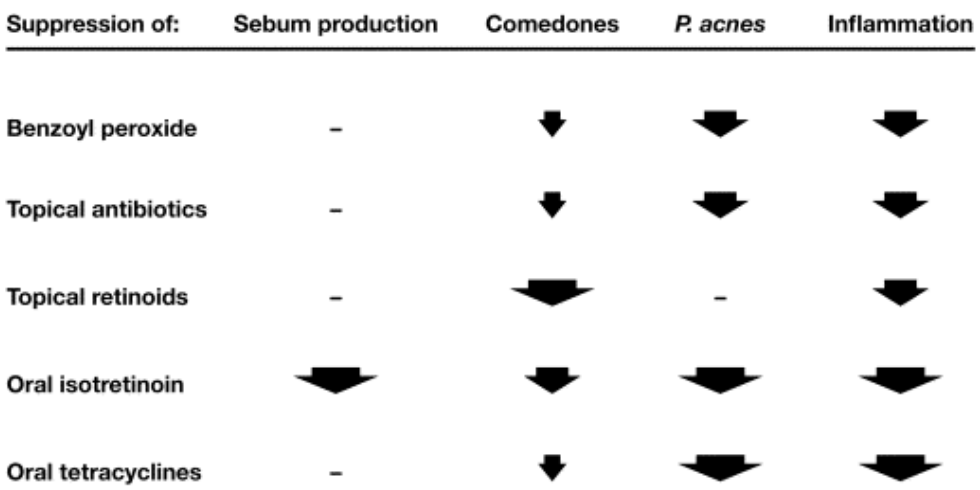


Table 1. Acne therapies and their associated activities.
(Reproduced from reference 14)

1.4 Combination of therapies for acne treatment

Unless acne is mild, effective treatment typically will include combinations of therapies. Clinical studies have assessed the efficiency and safety of combinations of components for acne treatment. These studies demonstrate significantly greater and faster results with the combination therapy than with the single agents alone. Because benzoyl peroxide is a potent bactericidal agent active against *P.* acnes - in addition to its ability to reduce the amount of FFA's - it has been combined with several other agents. However, benzoyl peroxide does not affect sebum production or composition as effectively as other classes of products do. Current approaches for mild to moderate acne treatment involve various combinations of agents such as topical antibiotics plus topical benzoyl peroxide, topical retinoids plus topical benzoyl peroxide, and topical retinoids plus topical benzoyl peroxide and antibiotics. These combination groups of agents and their activity are discussed below.

1.4.1 Combination of antibiotics plus benzoyl peroxide

A combination of the topical antibiotic clindomycin with benzoyl peroxide was reported to have an additive effect compared with monotherapeutic use of either agent.[20] Although both agents are effective against *P. acnes*, bacterial growth is only a single factor in the complex pathogenesis of acne. In terms of treating comedones, this combination is not perfect, because both benzoyl peroxide and antibiotics are only mildly comedolytic. In two of the studies completed by Dermik Laboratories Inc. there was a 36 and 58% reduction from baseline in total lesions in the clindamycin/benzoyl peroxide group, which was greater than the reductions seen in the benzoyl peroxide (28 and 52%) and clindamycin (15 and 42%) groups, respectively.[22] In evaluations conducted at the completition of two of the trials patients using clindamycin/benzoyl peroxide demonstrated significantly greater improvement than patients using benzoyl peroxide or clindamycin as a monotherapy. Figure 5 shows data assessed by physicians and presented below.[20,23]

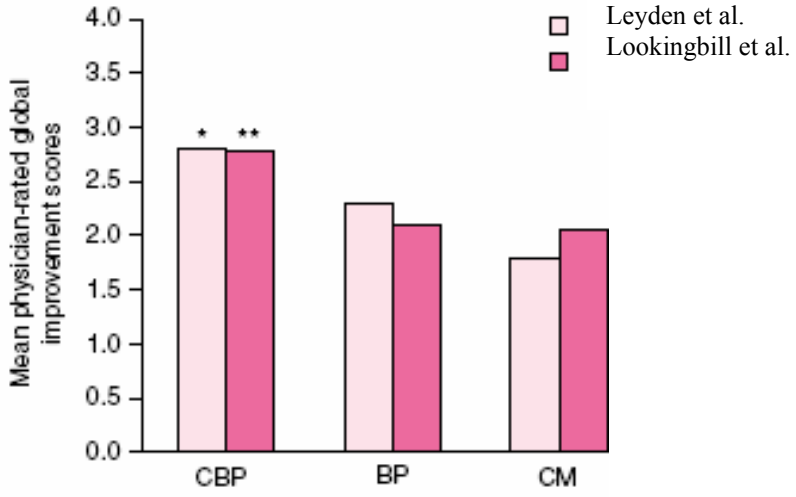


Figure 5. (Reproduced from references 20, 23) Mean physician rated global improvement scores of two randomized, parallel trials comparing clindamycin 1%/benzoyl peroxide 5% gel (CBP) with benzoyl peroxide 5% gel (BP) and clindamycin 1% gel (CM) in the treatment of patients with acne; Leyden et al.[23] and Lookingbill et al.[20] A 5-point rating scale (0 = worse to 4 = excellent improvement)

Similarly, in another study there was a greater number of patients who considered themselves "much better" after using clindamycin/benzoyl peroxide (34%) than benzoyl peroxide (25.6%) or clindamycin (14.6%).[24] Similar results were reported with a combination gel of erythromycin and benzoyl peroxide.[20]

1.4.2 Combination of retinoids plus benzoyl peroxide

The combination of a topical retinoid plus benzoyl peroxide provides an increasing effectiveness in acne treatment.

In an open study of 400 patients with moderate to severe acne, 88.1% of those receiving a combination of tretinoin and benzoyl peroxide achieved 80% to 90% clearing of acne lesions after 6 to 8 weeks of therapy.[14] In fact, the combination of tretinoin and benzoyl peroxide resulted in less irritation than when only tretinoin was used. The topical retinoid in this combination stimulates cellular turnover within pilosebaceous units and decreases cohesiveness of epidermal cells. The decrease in cellular cohesiveness results in decreased comedo formation, cell sloughing, and expulsion of existing comedones from the sebaceous follicles and a reduction in overall inflammation. Benzoyl peroxide also works as a comedolytic agent through its antibacterial action against *P. acnes.*

1.4.3 Treatment using topical retinoids plus topical benzoyl peroxide and antibiotics

Treatment with a topical retinoid and a benzoyl peroxide-antibiotic combination is usually described as sequential therapy rather than a combination treatment. The term sequential is used here because best results are obtained when one agent is applied in the morning and the other in the evening. A 21-day test study has shown that adapalene can be co-administered with benzoyl peroxide, clindamycin, and erythromycin with little or

no evidence of irritancy compared with significantly higher levels reported with similar tretinoin combinations.[14] Recent studies of Leyden and co-workers[14] showed that use of tretinoin and a combination of benzoyl peroxide and erythromycin (Benzamycin) has demonstrated superior effect than any of the 3 compounds used as monotherapy.

On the basis of clinical trial evidence, an algorithm for managing acne has been derived and is shown in Figure 6.

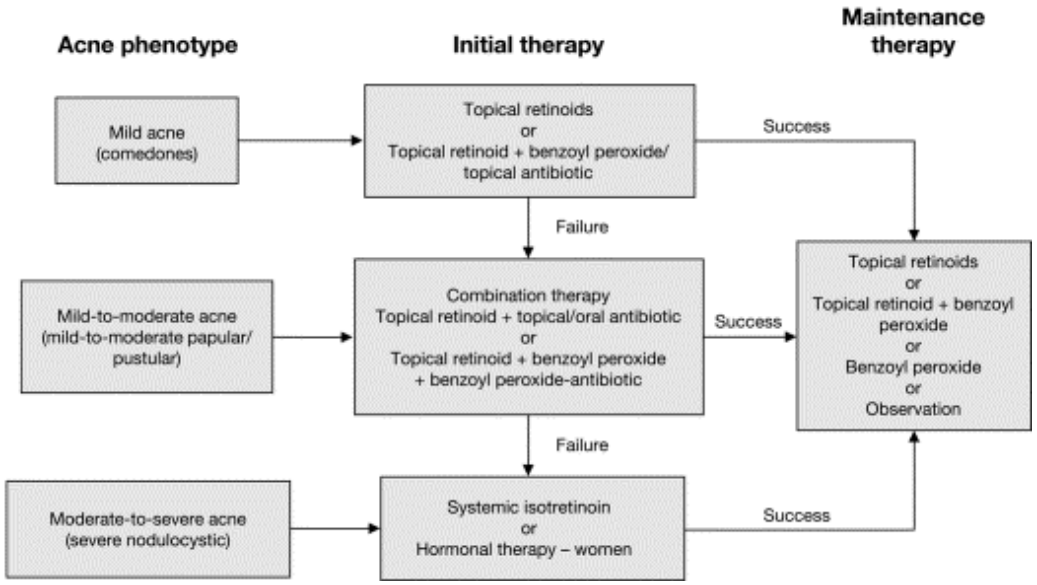


Figure 6. Algorithm for the management of acne vulgaris.
(Reproduced from reference 14)

1.5 Combinatorial chemistry

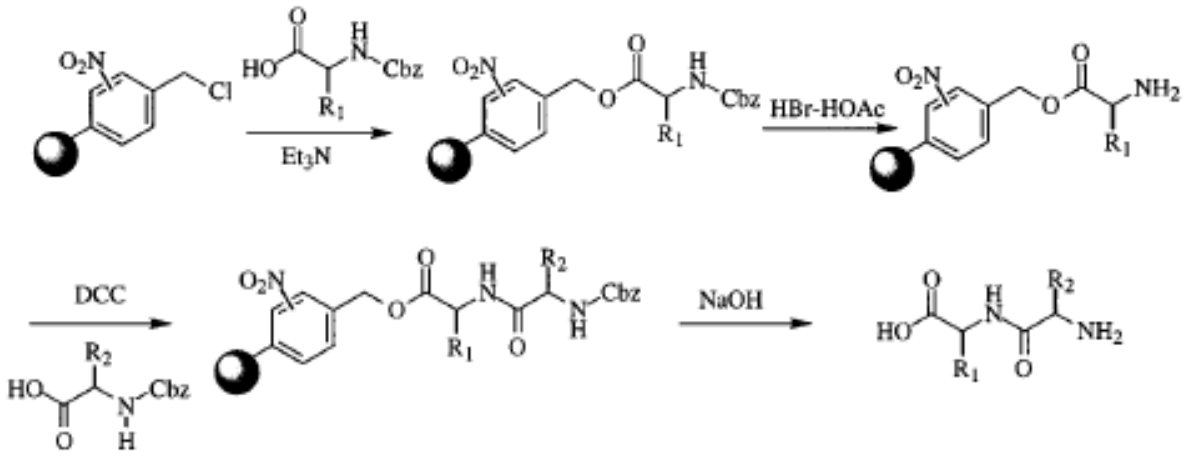
In the past few decades, combinatorial chemistry has become an extremely important tool in drug discovery. Today, combinatorial chemistry includes many strategies and processes for the fast synthesis of large, organized sets of compounds called libraries. Intelligently organized, combinatorial methods generate collections of molecularly diverse compounds that can be used for screening for biological activity. Combinatorial chemistry covers many research areas including new analytical methods, new computer modeling and database-related challenges, new synthetic approaches, new types of reagents, etc. The key of combinatorial chemistry is that a large range of analogues is synthesized using the same reaction conditions, the same reaction vessels and same time.[25,26] In this way there is an ability to synthesize many compounds at one time instead of making only one. Combinatorial chemistry approach has two phases:[27]

- making a library;
- finding an active compound;

1.6 Solid phase peptide synthesis

Combinatorial chemistry has its earliest origins in solid phase peptide synthesis. The term "solid phase peptide synthesis" was first introduced by Merrifield in 1963 to describe the preparation of a peptide on a polymer which remained insoluble throughout the synthesis.[28] He has received the Nobel Prize in 1984 for his work on solid phase peptide synthesis.[29] Merrifield's synthesis of a tetrapeptide is schematically shown below (Scheme 2).[30] Beginning with the attachment of N-protected amino acid to the solid support, the synthesis continued with the addition of further N-protected amino acids

until the desired chain was assembled. The peptide was then removed from its support by cleavage of the ester linkage.



Scheme 2. Merrifield's synthesis of a tetrapeptide.
(Reproduced from reference 30)

1.7 Combinatorial chemistry strategies

Combinatorial synthesis assembles building blocks in order to make new compounds. Collections of compounds to be screened are called libraries. Small libraries can be made as individual components by the techniques of parallel synthesis. Larger libraries can be made by assembling all possible combinations of a set of building blocks, and new lead compounds can be identified by using techniques like split-mix synthesis. The illustration of the principle of parallel synthesis is shown in the following Figure 7.

Figure 7 shows that different alcohols react with acid chlorides to form esters. A computer controls the additions of chemicals, which is done by robots, and a particular ester occupies a particular place in the matrix.

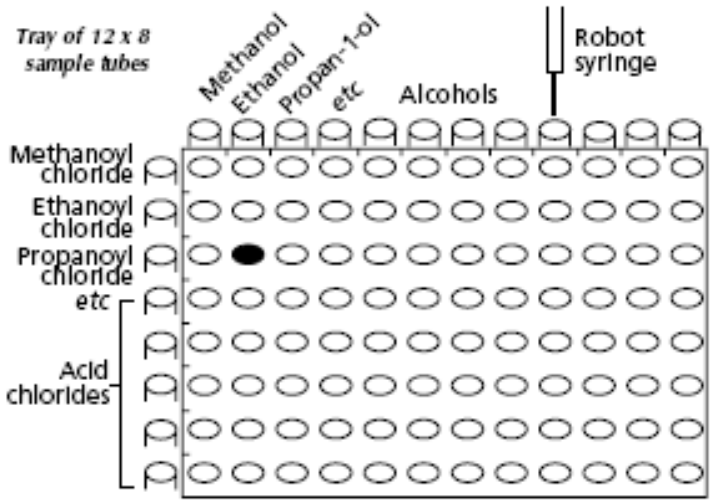


Figure 7. Combinatorial chemistry strategies: parallel synthesis
(Reproduced from reference 26)

The illustration of a split-mix synthetic technique is presented in Figure 8.

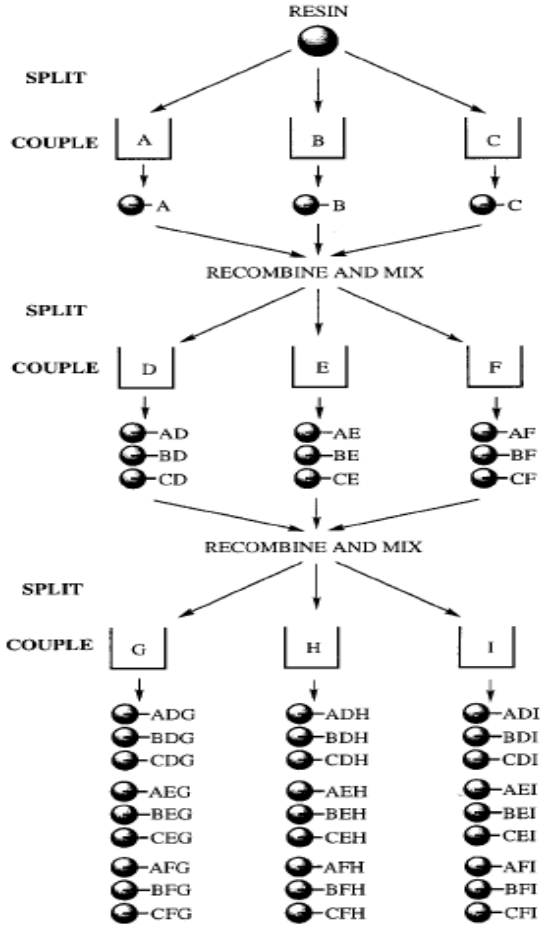
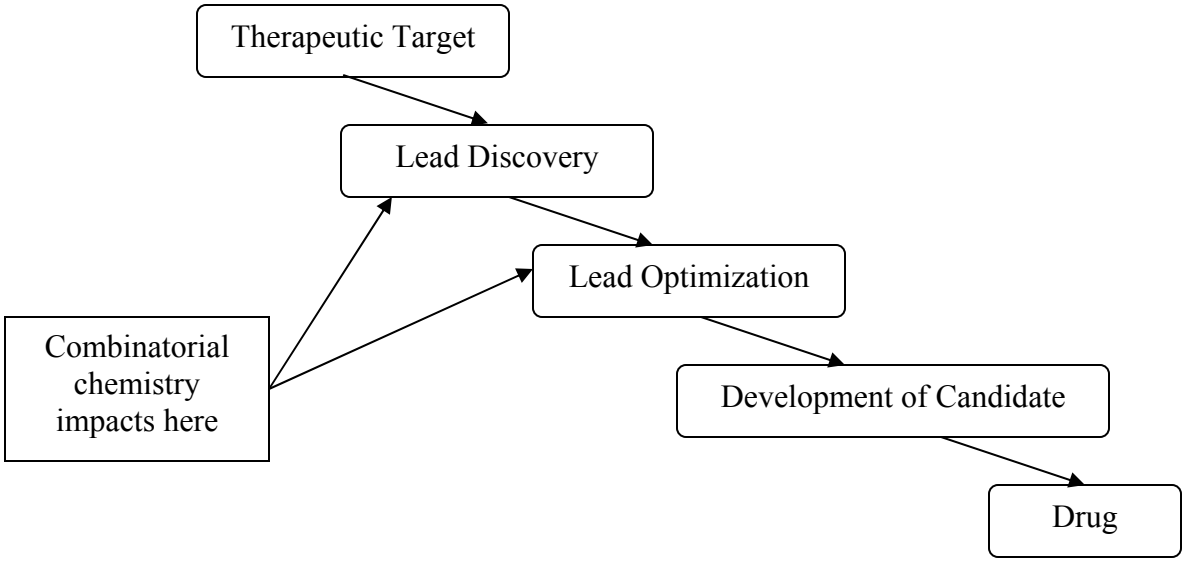


Figure 8. Combinatorial chemistry strategies: split-mix synthesis
(Reproduced from reference 30)

The example of split-mix synthesis shown in Figure 8 demonstrates three cycles, each with three reagents, leads to a library of 27 different compounds attached to the resin beads. The general rule is that with x monomers and n steps, we produce x^n compounds. There are difficulties with identifying the compounds since each bead contains a very little amount of compound (a few femtomoles).[31] Analytical techniques are used to determine the structure of compounds released from the beads. Mass spectrometry due to its sensitivity for very small amount of compounds is one of the most useful methods used to identify unknown substances.[31]

1.8 Drug discovery process

The new discipline of combinatorial chemistry has been a driving force for both pharmaceutical research and drug discovery. The outcome of the drug discovery process is the identification of a chemical structure that has desired potency against a specific biological target. Also, that structure has to have proper bioavailability and efficacy in an appropriate animal model of the targeted disease.[32] Drug discovery is a long and expensive process. It takes several years from starting a project to the point where a potential compound is submitted as a drug for development and clinical trials. At the first stage of the drug discovery process a lead compound (a structure with some degree of affinity) needs to be found. After the lead compound is identified, a second step begins which includes the identification of a drug development candidate by stepwise improvements of the lead structure. This process is illustrated in Scheme 3.[32]



Scheme 3. The key steps in the drug discovery process and the steps that can be influenced by the application of combinatorial chemistry. (Reproduced from reference 32)

1.9 References.

1. a)*Doctor's Guide; http://www.pslgroup.com/dg/337d6.html; 1/3/2001*; b) Pray, J.J., Pray, W.S. Teenages and acne: the role of pharmacist. *US Pharm.* **2003,** *28,* 6
2. Weiss, J.S. Current options for the topical treatment of acne vulgaris. *Pediatr Dermatol.* **1997,** *14*, 480-488.
3. http://thecesolution.com; Gossel, T.A. Topical prescription acne therapy: An overview.
4. Warner, G.T. and Plosker, G.L. Clindamycin/Benzoyl peroxide gel. A review of its use in the management of acne. *Am. J. Clin. Dermatol.* **2002,** *3*, 349-360
5. Eady, E.A., Cove, J.H. Is acne an infection of blocked pilosebaceous follicles? Implications for antimicrobial treatment. *Am. J. Clin. Dermatol.* **2000,** *1*, 201-209
6. Hunter, J.A.A., Savin J.A., Dahl M.V. Sebaceous and sweat gland disorders. In: *Clinical Dermatology,* 3rd ed. Malden: Blackwell Science Ltd; **2002,** 148-161.

7. a) Billow, J.A., Berardi R.R., DeSimone E. M., Newton G.D., et al. *Handbook of Nonprescription Drugs,* 13th ed. Washington DC: American Pharmaceutical Association; 2002: 777-791; b) Brown, S.K., Shalita, A.R. Acne vulgaris. *Lancet.* **1998,** *351*, 1871-1876; c) Eady, E.A. Bacterial resistance in acne. *Dermatology*, **1998,** *196*, 59-61. d) Baur, D.A., Butler, R.C.D. Current concepts in the pathogenesis and treatment of acne. *J. Oral Maxilolofac. Surg.* **1998,** *56*, 651-655.
8. Russell, J.J. Topical therapy for acne. *Am. Fam. Physician.* **2000,** *61 (2)*, 357-366.
9. Pillsbury, D.M. Acne, rosacea and miliaria: a manual of dermatology. Philadelphia (PA): W.B. Saunders Co., **1971:** 172-175
10. Yonkosky, D.M., Pochi, P.E. Acne vulgaris in childhood: Pathogenesis and management. *Dermatol. Clin.* **1986,** *4*, 127-136.
11. Ives, T.J. Benzoyl peroxide. *Am. Pharm.* **1992,** NS32, *8*, 33-38.
12. Turro, N.J. *Modern Molecular Photochemistry;* University Science Books: Mill Valley, CA, 1991.
13. Gibson, J.R. Rationale for the development of new topical treatments for acne vulgaris. *Cutis* **1996,** *57 (1S)*, 13-19.
14. Leyden, J.J. A review of the use of combination therapies for the treatment of acne vulgaris. *J. Am. Acad. Dermatol.* **2003,** *49, 3*, S200-S210.
15. Meynadier, J., Alirezai, M. Systemic antibiotics for acne. *Dermatology*, **1998,** *196*, 135-139
16. Mackrides P.S., Shaughnessy, A.F. Azelaic acid therapy for acne. *Am. Pham. Physician* **1996,** *54*, 2457-2459.

17. Graupe, K., Cunliffe W.J., Gollnick, H.P., Zaumseil, P.P. Efficacy and safety of topical azelaic acid (20 percent cream): an overview of results from European clinical trials and experimental reports. *Cutis* **1996,** *57* (1 suppl), 20-35.

18. Zander, E., Weisman, S. Treatment of acne vulgaris with salicylic acid pads. *Clin. Ther.* **1992,** *14*, 247-253.

19. Johnson, B.A., Nunley, J.R. Topical therapy for acne vulgaris. How do you choose the best drug for each patient? *Postgrad Med.* **2000,** *107*, 69-80.

20. Lookingbill, D.P., Chalker, D.K., Lindholm, J.S. Katz, H.I., Kempers, S.E., Huerter, C.J. Treatment of acne with a combination clindamycin/benzoyl peroxide gel compared with clindamycin gel, benzoyl peroxide gel and vehicle gel. *J. Am. Acad. Dermatol.* **1997,** *37*, 590-595.

21. Chalker, D.K., Shalita, A., Smith, J.G., Swann, R.W. A double-blind study of the effectiveness of a 3% erythromycin and 5% benzoyl peroxide combination in the treatment of acne vulgaris. *J. Am. Acad. Dermatol.* **1983,** *9*, 933-936.

22. Dermik Laboratories Inc. BenzaClin (TM) topical gel clindamycin 1% - benzoyl peroxide 5% gel: prescribing information. http://www.dermik.com

23. Leyden, J.J., Berger, R.S., Dunlap, F.E., et al. Comparison of the efficacy and safety of a combination topical gel formulations of benzoyl peroxide and clindamycin with benzoyl peroxide, clindamycin and vehicle gel in the treatments of acne vulgaris. *Am. J. Clin. Dermatol.* **2001,** *2(1)*, 33-39.

24. Tschen, E.H., Katz, H.I., Jones, T.M., et al. A combination benzoyl peroxide and clindamycin topical gel compared with benzoyl peroxide, clindamycin phosphate and vehicle in the treatment of acne vulgaris. *Cutis* 2001, *67(2)*, 165-169.

25. Pirrung, M.C. Molecular Diversity and Combinatorial Chemistry. Principles and applications. Elsevier, 2004.

26. http://www.combichemistry.com

27. Wilson, S.R., Czarnik, A.W. *Combinatorial chemistry. Synthesis and application;* A Wiley-Interscience Publication; New York, NY, 1997.

28. Merrifield R.B., Solid phase synthesis I. The synthesis of a Tetrapeptide. *J. Am. Chem. Soc.* **1963,** *85*, 2149-2154.

29. http://en.wikipedia.org

30. Gordon, K., Balasubramanian, S. Solid phase synthesis- designer linkers for combinatorial chemistry: a review. *J. Chemical Technology and Biotechnology* **1999,** *74*, 835-851

31. Lister, T. Chemistry now – Combinatorial chemistry. The Royal Society of Chemistry; http://www.combichemistry.com

32. Terrett, N.K. *Combinatorial chemistry.* Oxford University Press, 1998

CHAPTER 2. EXPERIMENTAL SECTION

2.1 Materials

Reagents and solvents were obtained from commercial suppliers and used as received unless otherwise noted. Alcohols for combinatorial chemistry experiments were also obtained from commercial suppliers. Methyl alcohol (Pharmco), ethyl alcohol (Aldrich), isopropyl alcohol (EMD), n-butyl alcohol (Acros Organics), 1-octanol (Aldrich), 2-octanol (Matheson Coleman & Bell), cyclohexyl alcohol (Acros Organics), benzyl alcohol (Baker Analyzed Reagent). Acetone was distilled over phosphorous pentoxide under argon. Tetrahydrofuran (THF) and dichloromethane (DCM) were dried under argon over sodium/potassium alloy, and calcium hydride, respectively. Ether and benzene were dried over sodium in argon atmosphere.

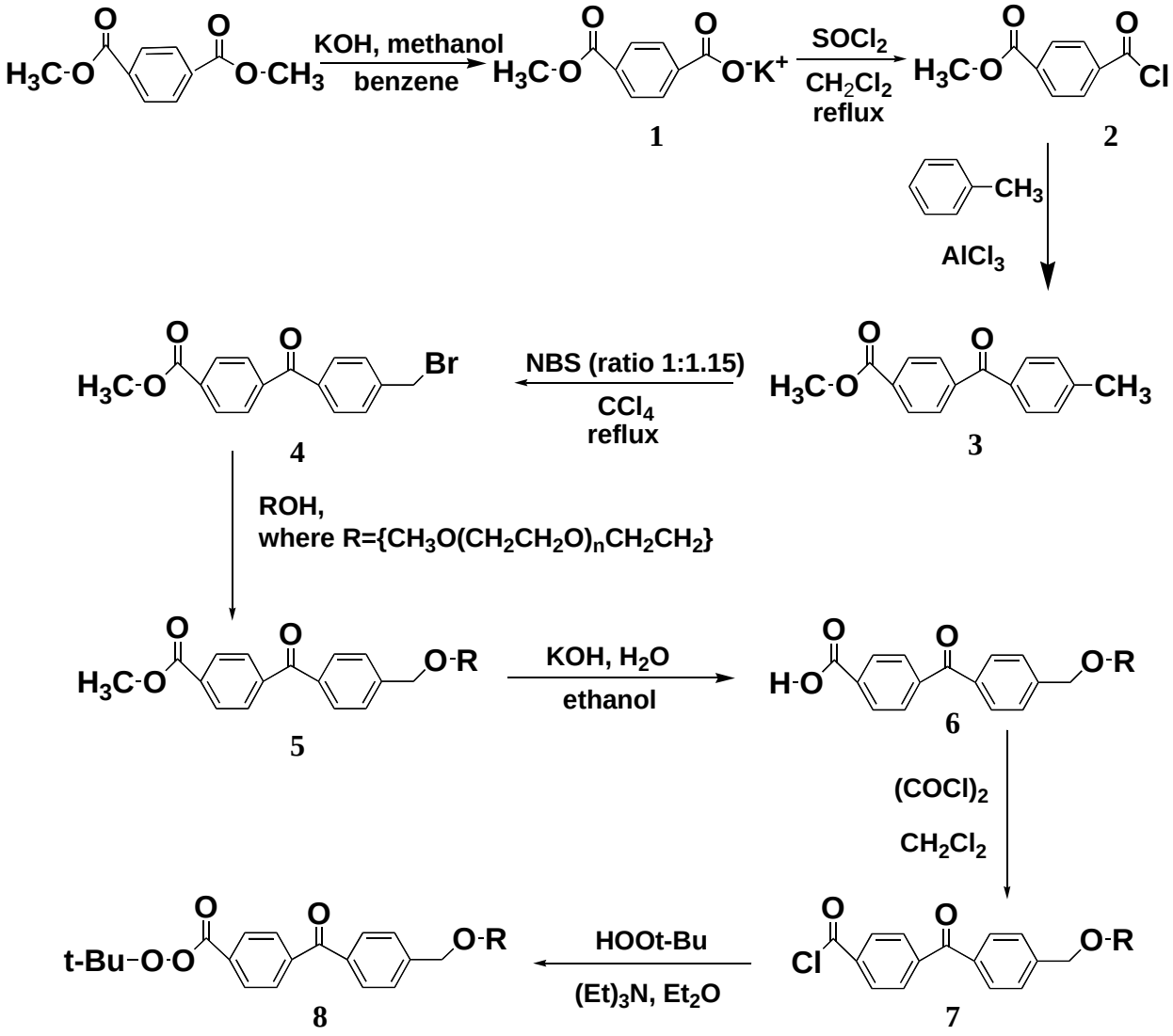
2.2 Instruments.

Melting points reported are uncorrected and were determined on a Thomas-Hoover capillary melting point apparatus. Infrared spectra were recorded using an IR 200 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian Gemini 200 MHz or Bruker AVANCE 300 spectrometer using $CDCl_3$ as a solvent. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) at 0.0 ppm for ^1H NMR. GC/MS data were collected using either Hewlett Packard 5988 mass spectrometer with a Hewlett Packard 5880A series gas chromatograph equipped with a 30cm x 0.25mm x 0.25 μm column or Shimadzu GC/MS-QP5050A mass spectrometer with a Shimadzu GC-17A equipped with 30cm x 0.25mm x 0.25 μm column. UV-visible spectra were obtained on Shimadzu UV-2401 PC spectrophotometers.

2.3 Synthesis of a multifunctional peroxyester

2.3.1 Synthetic scheme

A synthetic procedure for producing the desired multifunctional peroxyester **8** has been proposed and successfully completed. The synthetic sequence includes eight steps and is shown in Scheme 4.



Scheme 4. Scheme for synthesis of a peroxyester **8**.

2.3.2 Synthetic procedures

The procedures employed to obtain compounds are detailed below. The IR, ^{1}H and ^{13}C NMR spectra, as well as GC/MS are included in the Appendix B.

Potassium salt of the monomethyl terephthalate (1) was synthesized according to a procedure described earlier.[10] Reaction yield was 75%.

Terephthalic acid monomethyl ester chloride (2) was synthesized by a general procedure.[1,2] The reaction yield was 81%. Melting point (50-52 ^{o}C) and ^{1}H NMR (($CDCl_3$) δ = 3.98 (s, 3H), 8.15 (s, 4H aromatic protons)) are in a good agreement with the reported data.[2,3]

Methyl 4-(4-methylbenzoyl)benzoate (3). The Friedel-Craft acylation[4] reaction of **2** with toluene yielded 74% of **3**. Melting point was observed at 120-122 ^{o}C. ^{1}H NMR ($CDCl_3$) δ = 2.45 (s, 3H), 3.96 (s, 3H), 8.16-7.28 (4d, 8H aromatic protons); ^{13}C NMR ($CDCl_3$) δ = 21.7, 52.4, 129.1, 129.4, 129.6, 130.3, 133.0, 134.3, 141.8, 143.9, 166.3, 195.7.

Methyl 4-(4-bromomethylbenzoyl) benzoate (4). A solution of **3** (5.0g, $1.97x10^{-2}$mol) and N-bromosuccinimide (NBS) (3.83g, $2.17x10^{-2}$mol) and a trace amount of benzoyl peroxide in carbon tetrachloride (CCl_4) (30ml) was heated and stirred under reflux for 3 hours. The mixture was cooled to room temperature and solvent was subsequently evaporated in vacuo. Compound **3** was recrystallized from CCl_4 and pure white solid was obtained in 78% yield. ^{1}H NMR ($CDCl_3$) δ = 3.97 (s, 3H), 4.54 (s, 2H), 8.17-7.26 (4d, 8H aromatic protons); ^{13}C NMR ($CDCl_3$) δ =

Methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoate (5). A solution of methyl 4-(4-bromomethylbenzoyl) benzoate **4** (2 grams, $6x10^{-3}$ mol) was prepared in diethyleneglycol methyl ether (30ml). The solution was stirred during 48 hours at 90-100 ^{o}C. The reaction mixture was cooled to room temperature, combined with 50 mL of hexane and extracted with water (2x30ml). The organic layer was dried over sodium sulfate, and solvent was evaporated in vacuo. Purification was done by TLC over basic aluminum oxide with hexane/acetonitrile (5:1) as eluent and yielded 35% of **5** as a white solid with melting point 63-65 ^{o}C. ^{1}H NMR ($CDCl_3$) δ = 3.39 (s, 3H), 3.72-3.56 (m, 8H), 3.97 (s, 3H), 4.67 (s, 2H), 8.16-7.26 (4d, 8H aromatic protons); ^{13}C NMR ($CDCl_3$) δ = 52.5, 59.1, 70.0, 70.6, 70.7, 72.0, 72.6, 127.3, 129.5, 129.7, 130.3, 133.2, 136.2, 141.5, 143.9, 166.3, 195.6.

4-(4-((2-(2-Methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoic acid (6). A solution of **5** (0.2g, $5.92x10^{-4}$mol) and KOH (0.1g, $1.78x10^{-3}$mol) in ethanol (7 ml) and water (7 ml) was refluxed overnight. The solution was then acidified to pH=2 using concentrated hydrochloric acid under reflux and then the solution was poured onto a water/ice mixture. The aqueous layer was extracted with methylene chloride (2x10 ml). The combined organic layers were dried over magnesium sulfate, solvent was evaporated, and pure compound **6** was obtained in 84% yield, m.p. 95-97 ^{o}C. ^{1}H NMR ($CDCl_3$) δ = 3.4 (s, 3H), 3.73-3.57 (m, 8H), 4.67 (s, 2H), 8.24-7.26 (4d, 8H aromatic protons); ^{13}C NMR ($CDCl_3$) δ = 59.1, 70.0, 70.6, 70.7, 72.0, 72.6, 127.4, 129.8, 130.1, 130.3, 132.3, 136.1, 142.3, 144.0, 170.2, 195.6.

4-(4-((2-(2-Methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoyl chloride (7). A solution of **6** (0.085g, 2.37×10^{-4}mol) and one or two drops of dry dimethylformamide in dry methylene chloride (3 ml) was added dropwise to a solution of oxalyl chloride (0.06g, 4.75×10^{-4}mol) in methylene chloride (2 ml). The mixture was stirred overnight at room temperature in the dark, and subsequently the solvent was removed by evaporation in vacuo and compound **7** was obtained.

Methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) *t*-butyl perbenzoate (8). This perester was made using a previously described procedure.[5] ^{1}H NMR ($CDCl_3$) δ = 1.45 (s, 9H), 3.4 (s, 3H), 3.72-3.59 (m, 8H), 4.67 (s, 2H), 8.09-7.50 (4d, 8H aromatic protons); ^{13}C NMR ($CDCl_3$) δ = 26.3, 59.1, 70.0, 70.6, 70.7, 72.0, 72.6, 84.4, 127.3, 129.1, 129.9, 130.3, 130.7, 135.9, 141.9, 144.0, 163.7, 195.4.

2.4 Combinatorial synthesis

Combinatorial experiments were carried out at 40 ^{0}C in test tubes for 45 hours. 20 mg of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with 1 ml of different alcohols were used for the experiments. These alcohols are listed in Table 2.

#	Name of alcohol
1	Methanol, (99.9%)
2	Ethanol, (99.5%)
3	Butanol, (99%)
4	Isopropyl alcohol, (99.8%)
5	1-Octanol, (99.5%)
6	2-Octanol
7	Cyclohexanol, (98%)
8	Benzyl alcohol, (99.8%)

Table 2. Alcohols for combinatorial studies.

2.5 References.

1. Lookingbill, D.P., Chalker, D.K., Lindholm, J.S. Katz, H.I., Kempers, S.E., Huerter, C.J. Treatment of acne with a combination clindamycin/benzoyl peroxide gel compared with clindamycin gel, benzoyl peroxide gel and vehicle gel. *J. Am. Acad. Dermatol.* **1997,** *37*, 590-595.

2. Hotten, B.W. Metal terephthalamates a new class of oil-gelling agents. *Ind. Eng. Chem*. **1957,** *49*, 1691-1694.

3. Firestone, R.A. Total synthesis of β-lactam antibiotics. VI. 3-Arylcephalosporins. *J.Org.Chem.* **1974,** *39*, 3384-3387.

4. Smith, M.E. Friedel and Crafts' reaction. The carbomethoxybenzoyl chlorides with aromatic hydrocarbons and aluminum chloride. *J. Am. Chem. Soc.* **1922,** *43*, 1921.

5. Thijs, L., Gupta, S.N., Neckers, D. C. Photochemistry of perester initiators. *J. Org. Chem.* **1979,** *23*, 4123-4128.

CHAPTER 3. RESULTS AND DISCUSSION

3.1 Synthesis of multifunctional compound (8)

The compounds **potassium salt of the monomethyl terephthalate (1), terephthalic acid monomethyl ester chloride (2), methyl 4-(4-methylbenzoyl)benzoate (3), methyl 4-(4-bromomethylbenzoyl) benzoate (4)** were previously characterized and studied, while compounds **methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoate (5), 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoic acid (6), 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoyl chloride (7),** and **methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) *t*-butyl perbenzoate (8)** are new and being reported for the first time. There were several problems concerning some of the synthetic steps. Reaction of **3** with N-bromosuccinimide (NBS) was stopped with some amount of the starting compound remaining in the mixture because **methyl 4-(4-dibromomethylbenzoyl) benzoate** was detected by GC/MS. Purification of **(4)** was not performed and the crude mixture was used in the next step. There were some difficulties accomplishing nucleophilic substitution reaction of **4** with diethyleneglycol methyl ether and the subsequent purification of **5**. We conducted multiple attempts to carry out this reaction in the presence of different bases such as 1,8-diazabicyclo[2.2.2]octane (DBU), triethylamine (Et_3N), and sodium hydride (NaH), but no desired product was isolated. A successful way to execute this reaction in the absence of base is described in the experimental section. Purification of **5** by chromatography over silica gel failed because of the sensitivity of the ethylene glycol chain to the acidic medium. The best results were obtained using basic aluminum oxide with hexane/acetonitrile (5:1) as eluent. Pure **5** was

confirmed by GC/MS, ^{1}H NMR, decoupled ^{13}C NMR. According to GC/MS (Figure 4, Appendix B) the molecular ion peak at m/e 372 and subsequent fragmentation are in agreement with the assigned structure. The ^{1}H NMR spectrum (Figure 6, Appendix C) was interpreted as follows: two singlets at 3.39 and 3.97 were assigned to the methyl hydrogens of ether and ester groups, respectively; the singlet at 4.67 was assigned to the two hydrogens of the methylene group attached to the benzene ring; eight hydrogens of two benzene rings appeared as four doublets at 8.16-7.46 ppm; eight hydrogens of the diethyleneglycol methyl ether chain appeared as a multiplet at 3.72-3.56. A literature comparison for the glycol chain hydrogens of a similar type as those in **5** is found in the spectrum of (2S)-1-benzyloxy-2-(2-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}ethoxy) propane (Figure 9), which was described by Meijer and co-workers.[1]

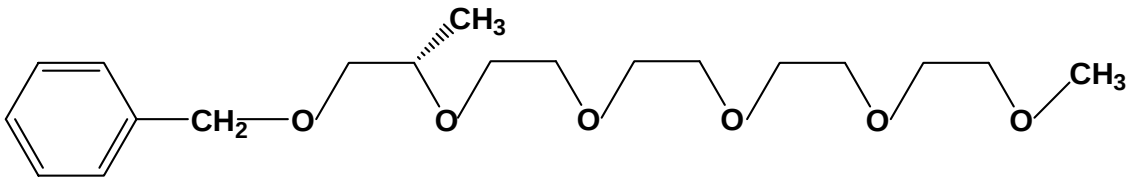


Figure 9. Structure of (2S)-1-benzyloxy-2-(2-{2-[2-(2-methoxyethoxy) ethoxy]ethoxy}ethoxy)propane.

(2S)-1-Benzyloxy-2-(2-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}ethoxy)propane, which has 19 secondary and tertiary hydrogens in its glycol chain, shows a multiplet in its ^{1}HNMR spectrum at 3.71-3.37 ppm nearly exactly like those in the spectrum of **5**.[1] This similarity is cited as confirmation for our structural assignment and shown in Figure 10.

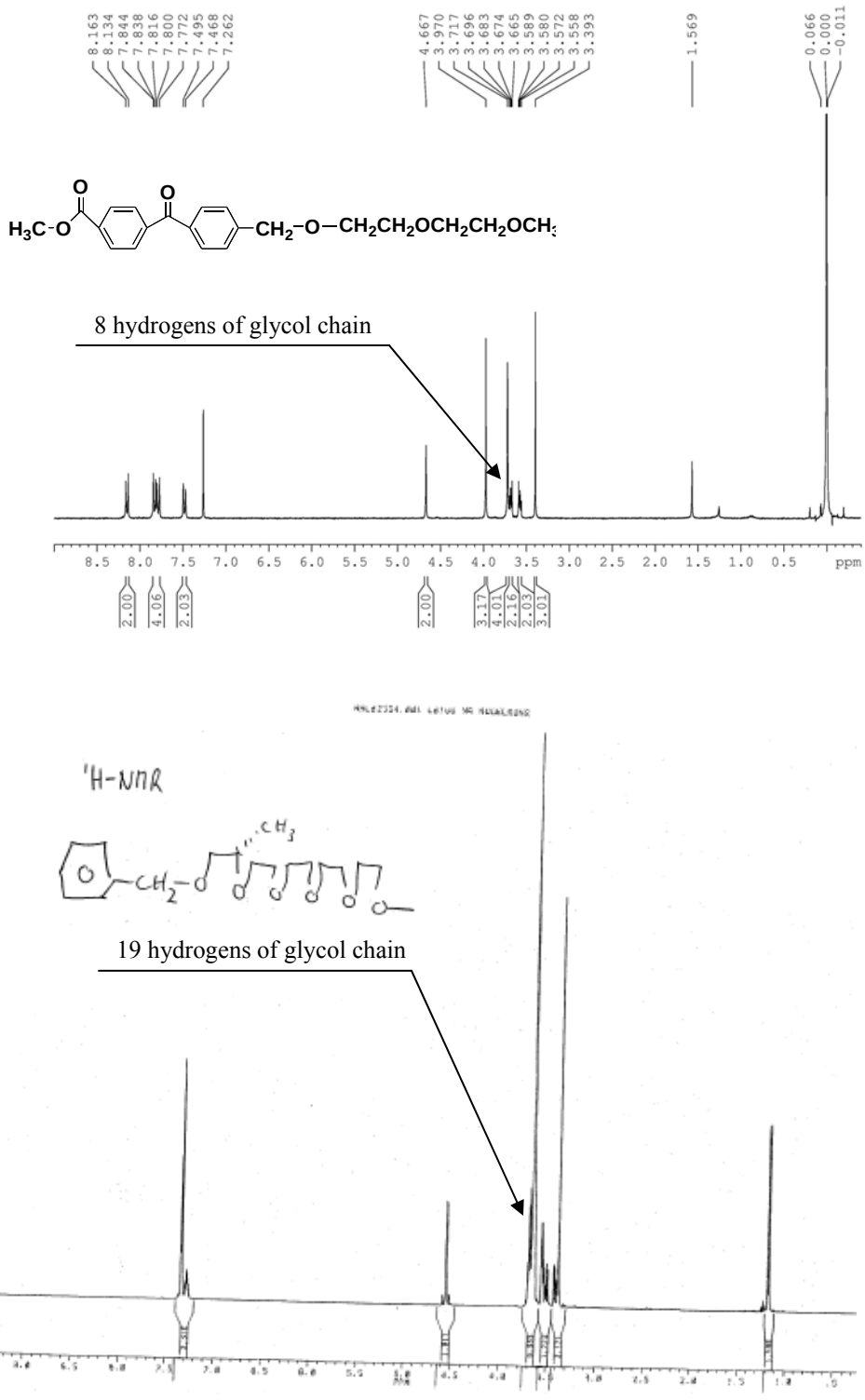


Figure 10. [1]H NMR spectra of **5** (a) and (2S)-1-Benzyloxy-2-(2-{2-[2-(2-methoxyethoxy)ethoxy]ethoxy}ethoxy)propane (b).
Spectrum of (b) reproduced from reference 1

Another way to confirm the structural assignment of **5** was comparison of its ^{13}C NMR spectrum (region for glycol chain) with that of di(ethyleneglycol)methyl ether, which was a precursor of **5.** The comparison of these spectra are presented in Figure 11.

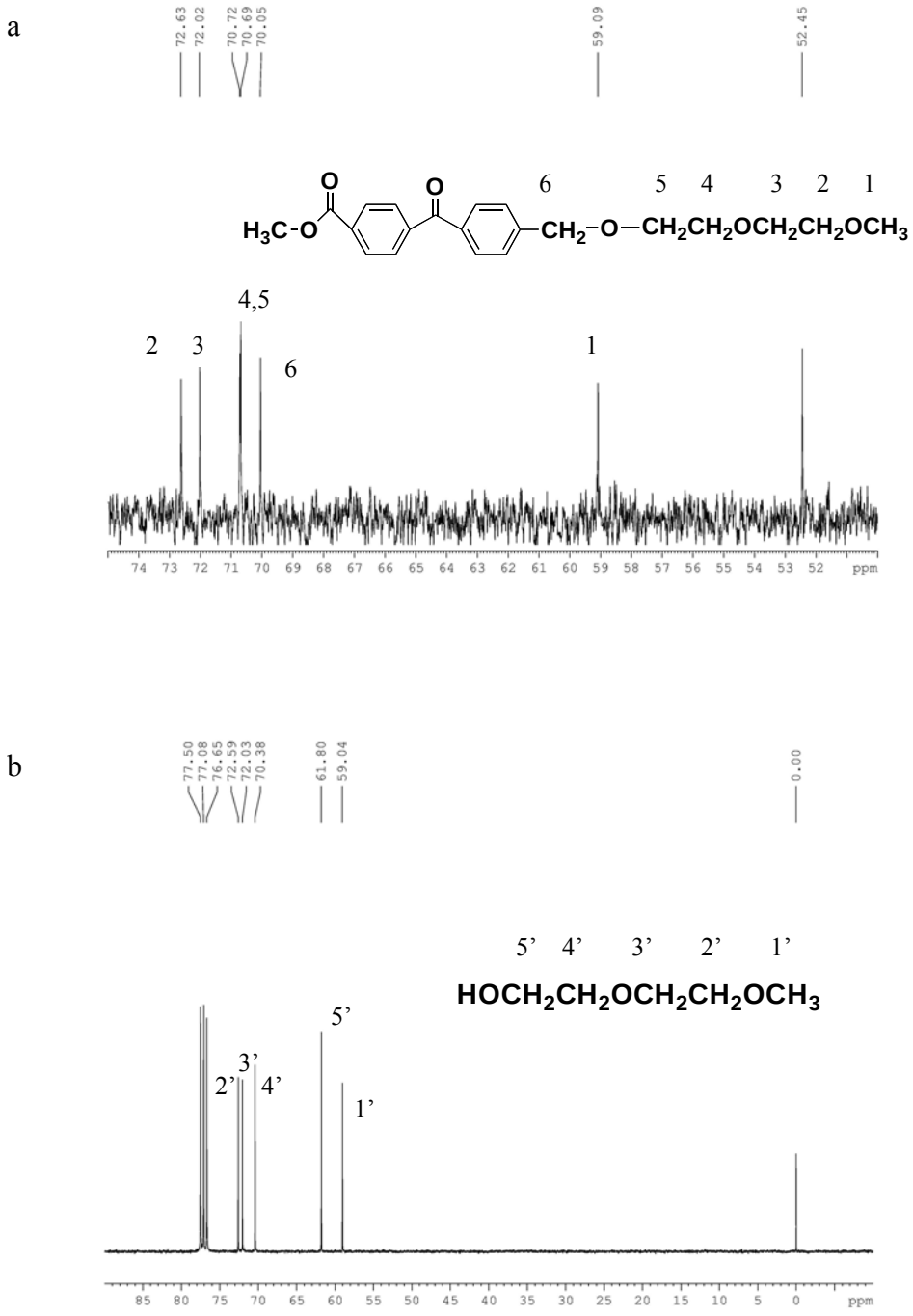


Figure 11. ^{13}C NMR spectra for glycol region of **5** (a) and di(ethyleneglycol)methyl ether (b).

The carbons labeled 1,2,3, and 4 in ether chain of **5** have essentially identical chemical shifts with the carbons labeled 1', 2', 3' and 4' in the di(ethyleneglycol)methyl ether, respectively. Carbons 5 and 5' in the two compounds are not expected to be identical, and indeed carbon 5 in **5** is shifted downfield compared to its counterpart 5' due to proximity with the aromatic ring of the benzyl group.

The structural assignment for 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoic acid (6) was confirmed by ^{1}H NMR, decoupled ^{13}C NMR, and IR spectroscopy. ^{1}H NMR Figure C9 (Appendix C) shows that aromatic hydrogens have shifted downfield which is characteristic for carboxylic acids.[2] The only difference between **5** and **6** as expected was the disappearance of the singlet peak at 3.97 which corresponded to methyl ester hydrogens. Its infrared spectrum Figure C11 (Appendix C) shows a C=O stretching band of the carboxylic acid at 1689 cm^{-1} which is typical for this kind of compounds.[2] The IR spectrum of benzoic acid shown in Figure 12 for comparison purposes.[3] It shows an identical absorption at 1689 cm^{-1}.

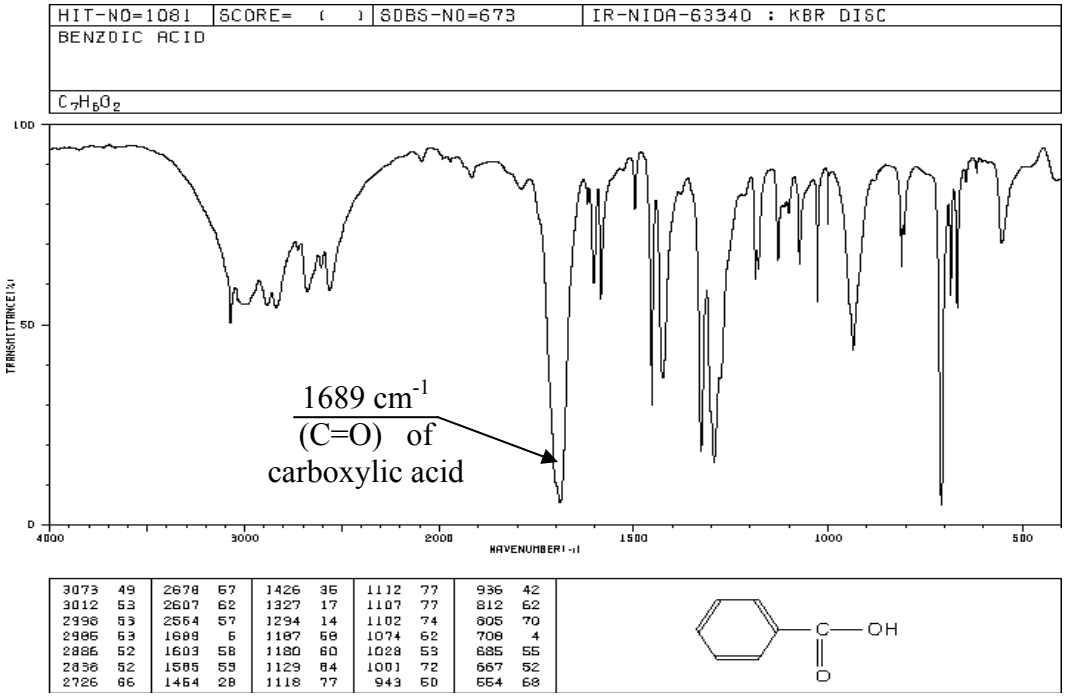


Figure 12. IR spectrum of benzoic acid.
Reproduced from reference 3

The other carbonyl absorption at 1647 cm^{-1} was assigned to the carbonyl group of benzophenone moiety which is in good agreement with literature data.[3,2]

Rapid hydrolysis of the **4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) benzoyl chloride (7)** to the corresponding acid represented another challenge. The hydrophilic nature of the diethyleneglycol methyl ether could possibly explain the fast hydrolysis of **7**. To avoid the undesired hydrolysis, compound **7** was used in the next reaction immediately after evaporation of the solvent; however, an IR taken right after compound **7** was obtained shows disappearance of carbonyl group of acid and new stretching vibration at 1784 cm^{-1}, which is in good agreement with literature data for acid chlorides.[2] A weak band at 1723cm^{-1} appearing in the spectrum probably results from Fermi resonance between the C=O band and the overtone of a lower wavenumber band near 875 cm^{-1}.[2] Figure 13 compares an IR spectra of **6** and **7**.

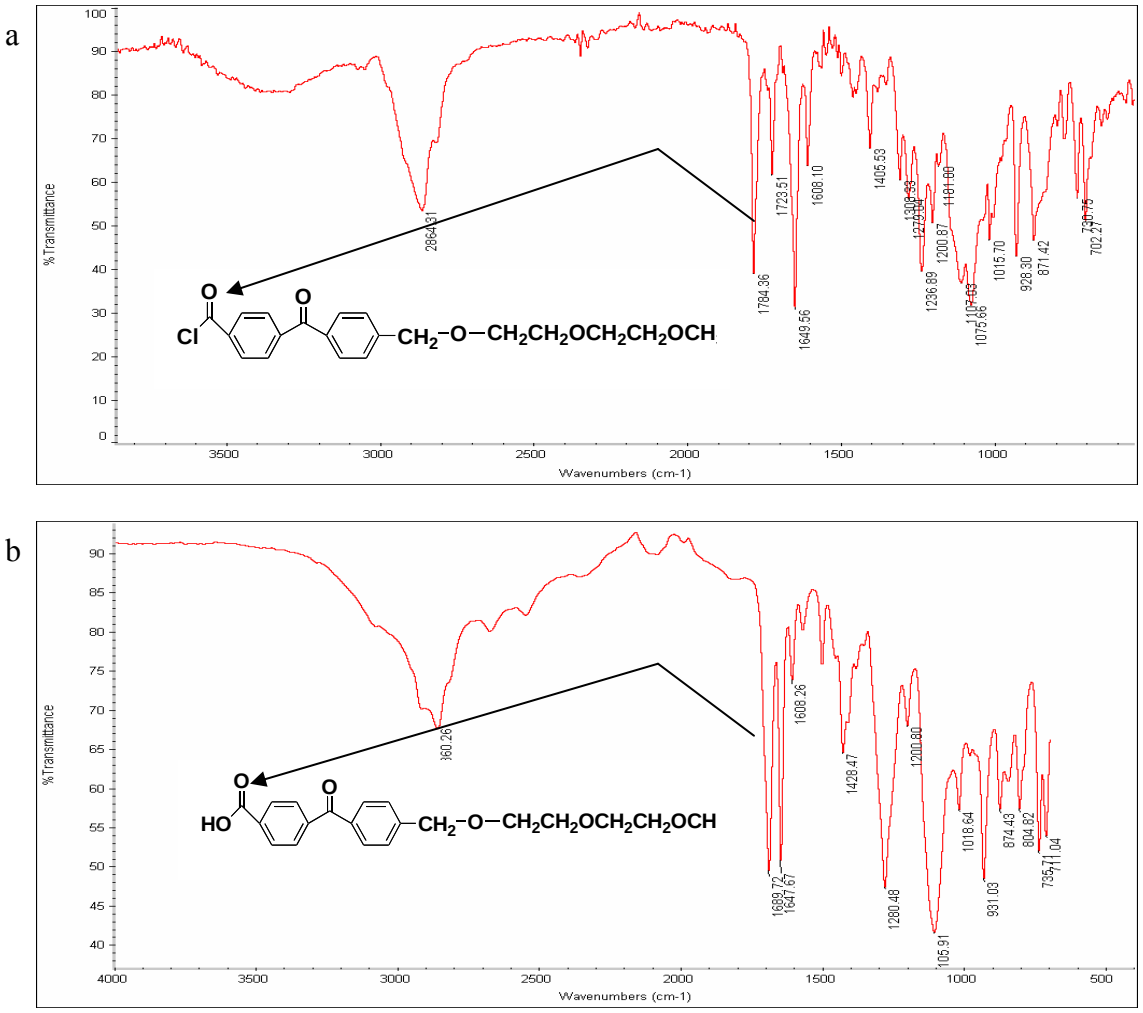


Figure 13. IR spectra of **6** (a) and **7** (b).

The ^{1}H NMR of **methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) *t*-butyl perbenzoate (8),** in contrast to its precursors **5**,**6**,**7**, was expected to show a peak corresponding to *t*-Bu group hydrogens. Compound **8** was synthesized three times and same spectroscopic data were observed at all times. Appearance of 9 hydrogens of *t*-Bu group of **8** was indeed observed at 1.44 ppm. We have confirmed from literature data for

similar compounds that *t*-Bu group hydrogens appear at 1.42 ppm in *tert*-Butyl peroxybenzoate (Figure 14).[4]

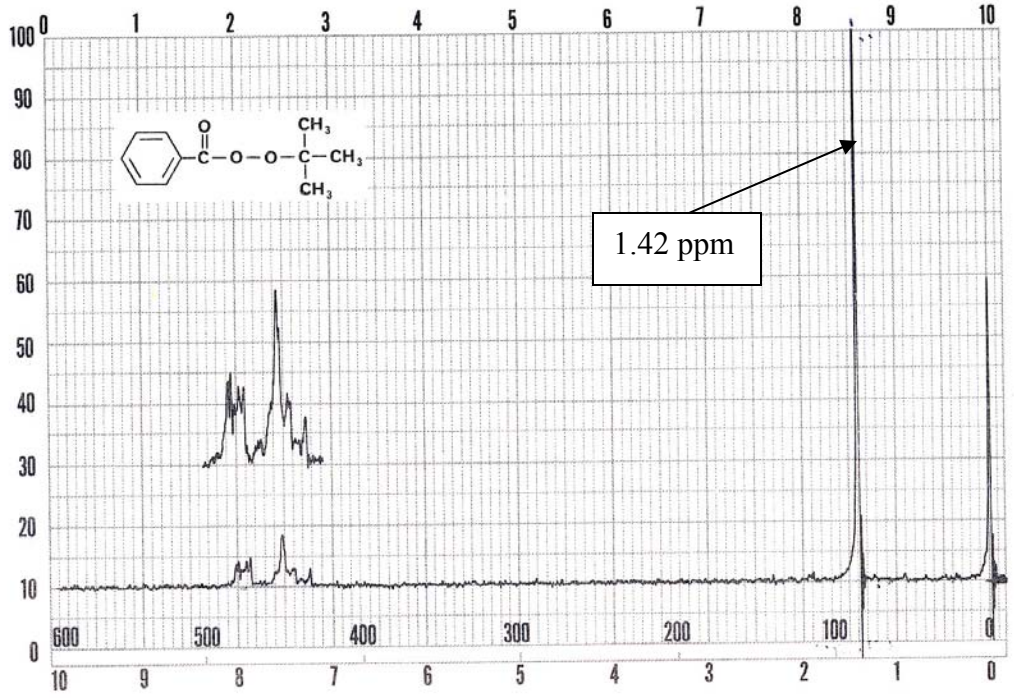


Figure 14. ^{1}H NMR spectrum for *tert*-Butyl peroxybenzoate.
Reproduced from reference 4

This comparison excludes the possibility of having a *t*-Bu ester group, whose protons appear at 1.59.[3] The ^{1}H NMR of *tert*-Butyl benzoate is shown in Figure 15 for comparison.[3]

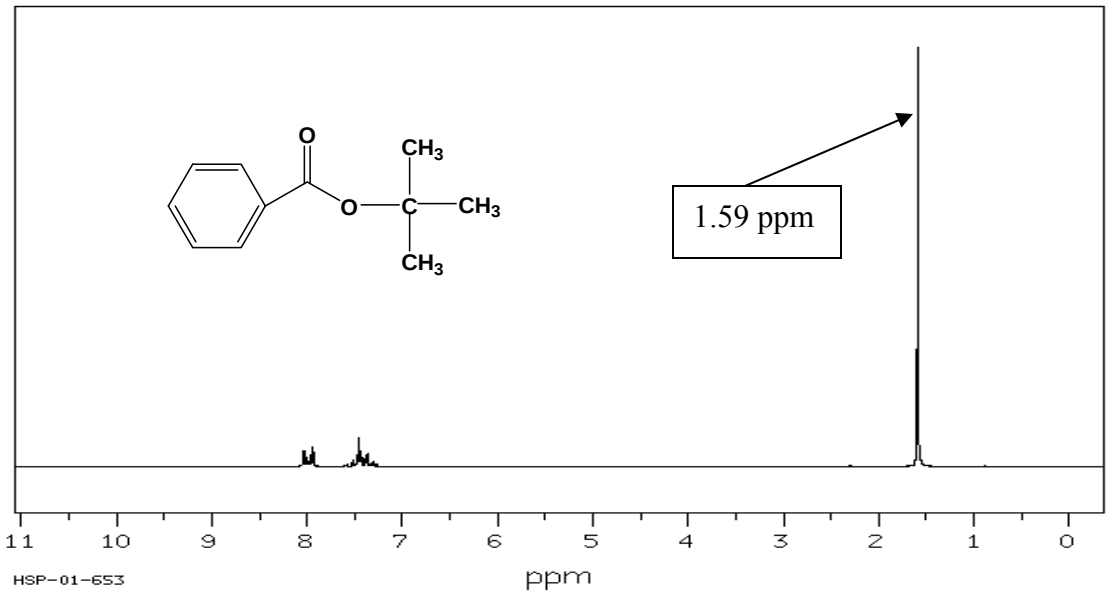


Figure 15. ^{1}H NMR spectrum for *tert*-Butyl benzoate.
Reproduced from reference 3

The UV-vis absorption spectrum of **methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) *t*-butyl perbenzoate (8)** in HPLC grade acetonitrile is shown in Figure 16. It displays the characteristic $n \rightarrow \pi^*$ and $\pi \rightarrow \pi^*$ absorptions of the benzophenone chromophore with maxima at 348 and 256 nm, respectively.[5]

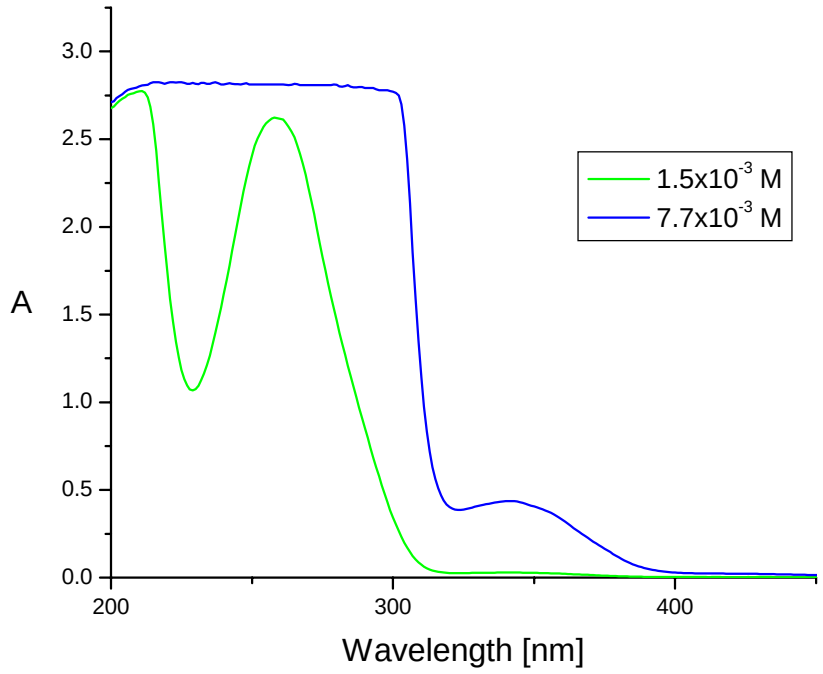
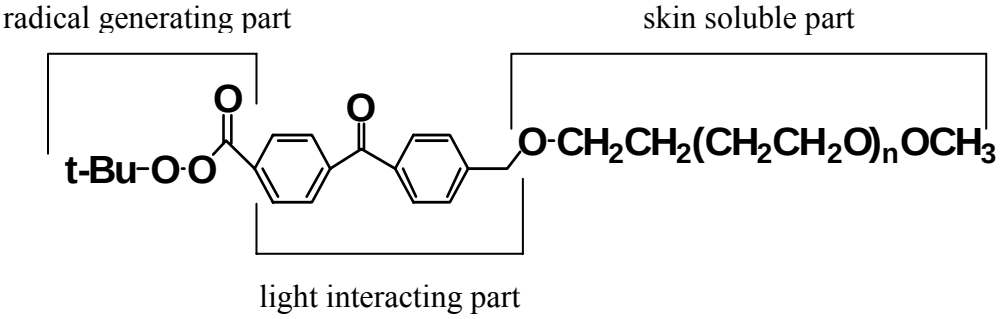


Figure 16. UV-vis absorption spectra of perester **8** in acetonitrile.

Summarizing, in this work we have synthesized and characterized a new multifunctional peroxyester, which may have improved potential for efficient treatment of acne and other skin diseases. The structure features of the compound are given below.



Scheme 5. Schematic representation of a multifunctional compound.

The molecule contains three parts, which are designed to serve different functions. A *tert-Bu* peroxide moiety is an essential radical generating part for acne treatment. Recent studies showed that thermal stabilities of dialkyl peroxides and alkyl peroxyesters are higher than diacyl peroxides such as benzoyl peroxide.[6] The higher stability of these peroxides prevents premature degradation of the compounds and leads to longer antimicrobial effects.[6] Comparison of the biological activities of the compounds with a *tert-Bu* peroxide moiety with "control" compounds without such a peroxide demonstrated that the oxidizing properties of the peroxides contributed significantly to antibacterial action.[7] The benzophenone chromophore allows one to photochemically control radical generation and ensures the selectivity of radical formation. We have chosen the benzophenone moiety since it has been extensively studied and its behavior is well understood. Finally, the diethyleneglycol methyl ether chain of the molecule ensures better skin affinity, which is an essential requirement for the acne treatment agents. We attached diethyleneglycol methyl ether chain to the benzophenone moiety to enhance its solubility in water and to explore interactions between antimicrobial agents and bacteria cell membranes.

3.2 Combinatorial studies

A brief combinatorial study using parallel synthesis methodology was conducted by using eight different alcohols and methyl 4-(4-bromomethylbenzoyl) benzoate **4** as the substrate. For these combinatorial reactions, substrate 4 had to be very pure, unlike its use in the reaction to produce the multifunctional peroxyester **8**, discussed previously. Several solvent systems were used in attempts to purify **4** via column chromatography.

Due to the close R_f values of **4** and its dibromo derivative this methodology did not result in good separation. The most successful way which allowed us to obtain 99.9% pure **4**, was double recrystallization from cyclohexane followed by recrystallization from a hexane/dichloromethane (8/2) mixture. Figure 17 shows the GC/MS of **4** and a single peak on gas chromatography verifies its 99.9% purity.

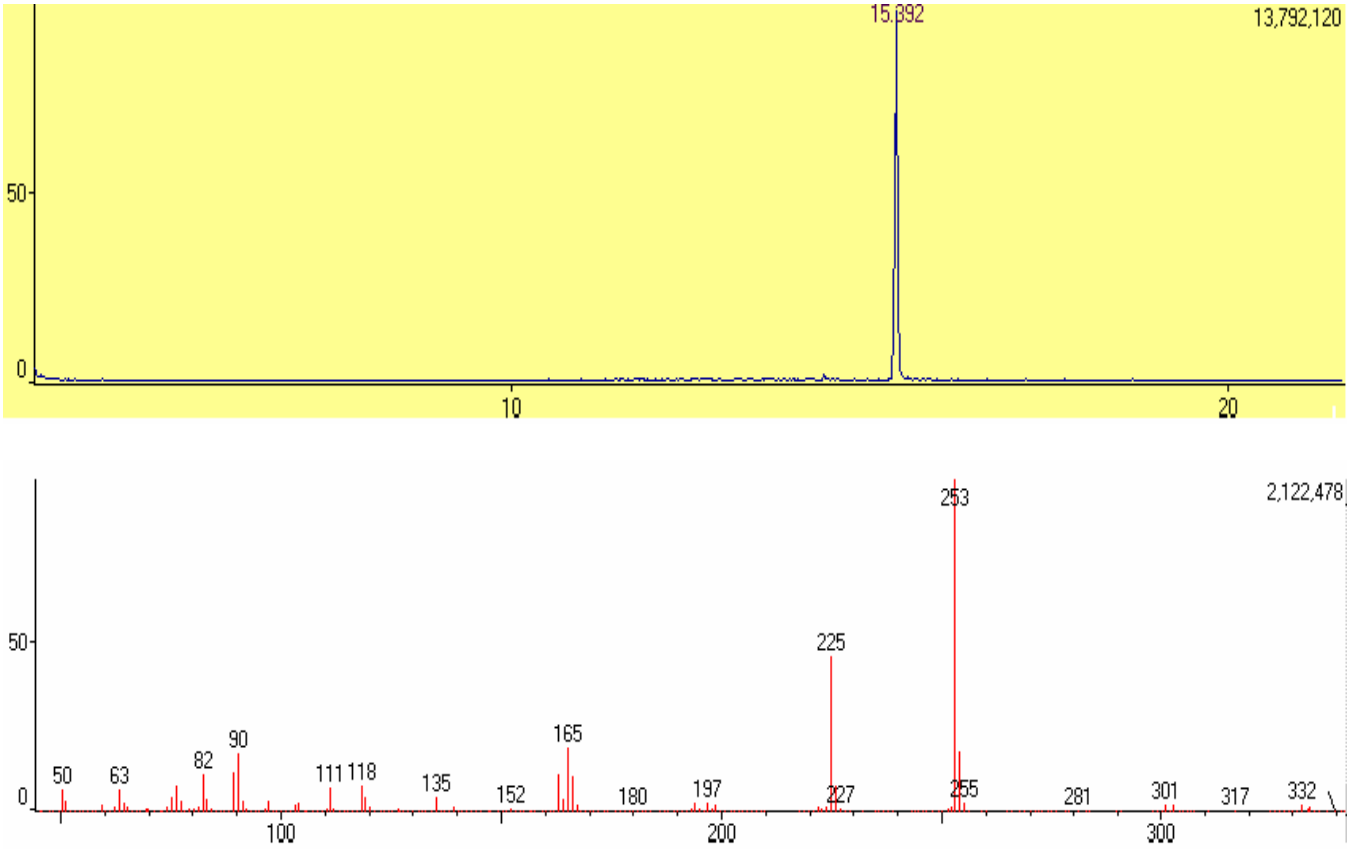


Figure 17. GC/MS of methyl 4-(4-bromomethylbenzoyl) benzoate **4** .

Results of reactions of methyl 4-(4-bromomethylbenzoyl) benzoate **4** with different alcohols are discussed below. Two side products were observed in each reaction

in addition to the desired product of nucleophilic substitution. One of the side products (Side product A) showed a molecular ion at 254 and MS fragmentation consistent with the structure of methyl 4-(4-methylbenzoyl)benzoate (Figure 18a). The other side product (Side product B) had 268 for its molecular ion peak in the mass spectrum and the probable structure is shown in Figure 18b.

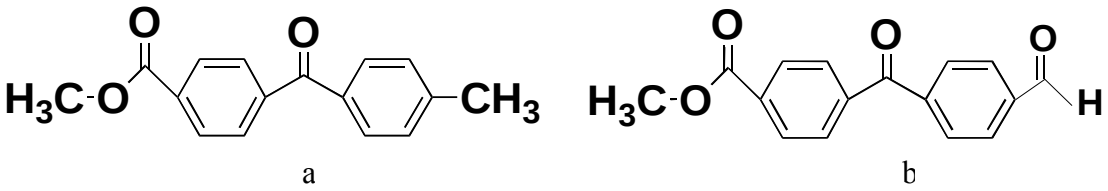


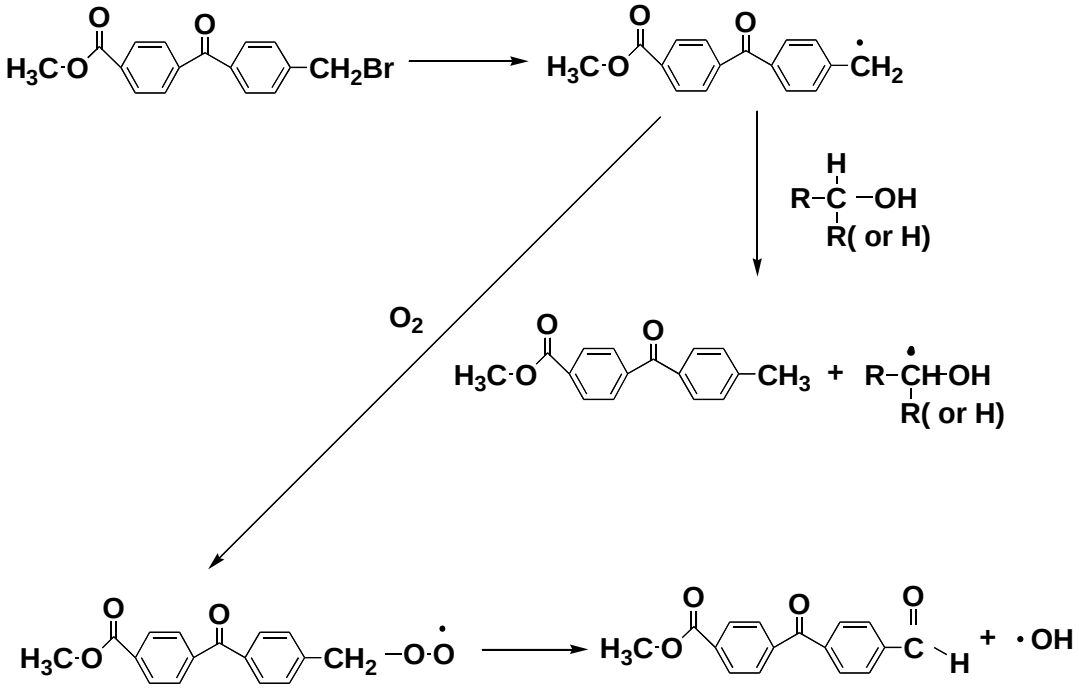
Figure 18. Structures of the side products.
(a) side product A, (b) side product B

Product ratios for all experiments are summarized in the table below.

Name of alcohol	% of Side product A	% of Side product B	% of Product of nucleophilic substitution reaction
Methanol, (99.9%)	3.2	11.6	60
Ethanol, (99.5%)	13	3.2	36
Butanol, (99%)	17	15	17
Isopropyl alcohol, (99.8%)	16.2	12.7	10.3
1-octanol, (99.5%)	13.9	N/A	N/A
2-octanol	33	21.7	N/A
Cyclohexanol, (98%)	8.5	16.7	7.6
Benzyl alcohol, (99.8%)	9.9	17.6	10

Table 3. Ratios of products in combinatorial experiments

No experiments designed to investigate the mechanisms responsible for the formation of these compounds were undertaken, but it is obvious that they are oxidation and reduction products of the starting benzyl alcohol moiety. A free radical mechanism seems most likely, shown in Scheme 6, but is unproven. Oxygen was not excluded from these reactions during a 45 hour heating period at 40^0. These preliminary data suggest that a controlled reaction condition study should be pursued in further research studies on this topic.



Scheme 6. Possible origin of side products in combinatorial synthesis reaction.

3.3 References

1. Brunsveld, L., Zhang, H., Glasbeek, M., Vekeman, J.A., Vekeman, J.M., Meijer, E.W. *J. Am. Chem. Soc.* **2000,** *122*, 6175-6182.

2. Silverstein, R. M., Webster, F.X. Spectrometric Identification of Organic Compounds, 6th ed. John Wiley & Sons, Inc., USA p. 90-100; 217-248.

3. SDBS (Spectral Database for Organic Compounds) http://www.aist.go.jp

4. Pouchert, C.J., The Aldrich Library of NMR Spectra, 2nd ed. Volume 2, p.281.

5. Shah, B.K., Neckers, D.C. Triplet energy distribution in photoinitiators containing two dissociable groups. *J.Org. Chem.* **2002,** *67*, 6117-6123.

6. Klenk, H., P. H. Götz, and R. Siegmeier **1991,** Peroxy compounds, organic, p. 200-218. *In* B. Elvers, S. Hawkins, and G. Schulz (ed.), Ullmann's Encyclopedia of Industrial Chemistry, 5th ed. VCH Publishers, Weinheim, Germany.

7. Liu, W., Liu, X., Knaebel, D., Luck, L., and Li, Y. *Antimicrobial Agents and Chemotherapy* **1998,** 911-915.

CHAPTER 4. CONCLUSIONS

A new multifunctional compound, **methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) *t*-butyl perbenzoate (8),** has been synthesized and characterized by ^{1}H NMR, decoupled ^{13}C NMR and UV-vis spectra. The new multifunctional organic peroxyester is suggested to be a potential agent for acne treatment purposes. Precursors of **8**, namely **5,6,7** also have never been reported before and their structures were confirmed by different available techniques such as IR,GC/MS, ^{1}H NMR, decoupled ^{13}C NMR.

Combinatorial chemistry experiments were conducted for eight different alcohols. Results showed that by increasing the chain length in the alcohols, the amount of product of nucleophilic substitution reaction was decreased. According to GC/MS there were two side products in addition to the major product in most of the reactions which exhibited ions at m/e 254 and m/e 268.

CHAPTER 5. FUTURE DIRECTIONS OF THE RESEARCH

Research in the area of acne treatment has been done throughout decades, however, still remains attractive, interesting and challenging. A future direction of this work is the *in vitro* studies of the antibacterial activity of this newly synthesized, multifunctional organic peroxyester. Photochemical properties of methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy)methylbenzoyl)) *t*-butyl perbenzoate (8) can be studied in order to expand knowledge of photochemical properties of that class of compounds.

Potential ways of tail functionalization using peptide chain and other biological molecules instead of diethyleneglycol methyl ether can also be considered as future application of this research.

APPENDIX A

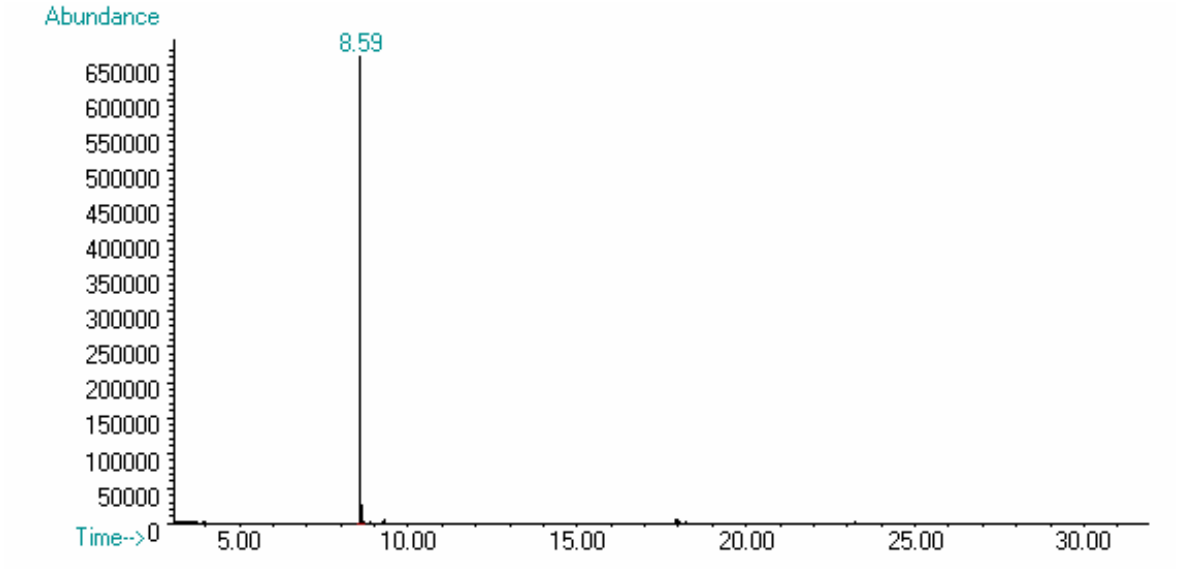
LIST OF ABBREVIATIONS:

DHEAS	Dehydroepiandrosterone sulfate
P. acnes	Propionibacterium acnes
BP	Benzophenone peroxide
FFA	Free fatty acids
Cbz	Benzyloxycarbonyl
DCC	1,3- Dicyclohexylcarbodiimide
MHz	MegaHertz
ppm	parts per million
NMR	Nuclear magnetic resonance
s (for NMR)	Singlet
d (for NMR)	Doublet
m (for NMR)	Multiplet
ml	Milliliter
g	Gram
mol	Mole
mmol	Millimole
mp	Melting point
^{0}C	Degree Celsius
K	Kelvin
TLC	Thin Layer Chromatography
GC	Gas Chromatography

MS	Mass Spectrophotometry
UV	UltraViolet
IR	InfraRed
THF	Tetrahydrofuran
NBS	N-bromosuccinimide
CCl_4	Carbon tetrachloride

APPENDIX B

Figure B1. Gas chromatogram and mass spectra of the terephthalic acid monomethyl ester chloride **(2)**.



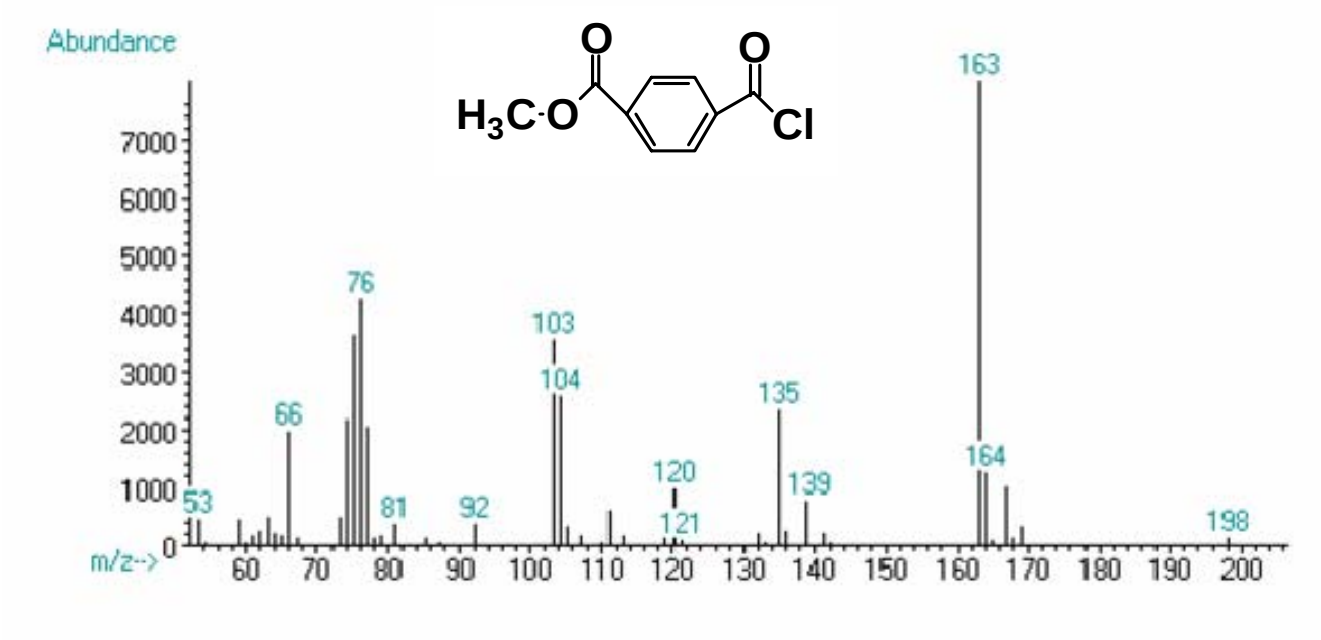
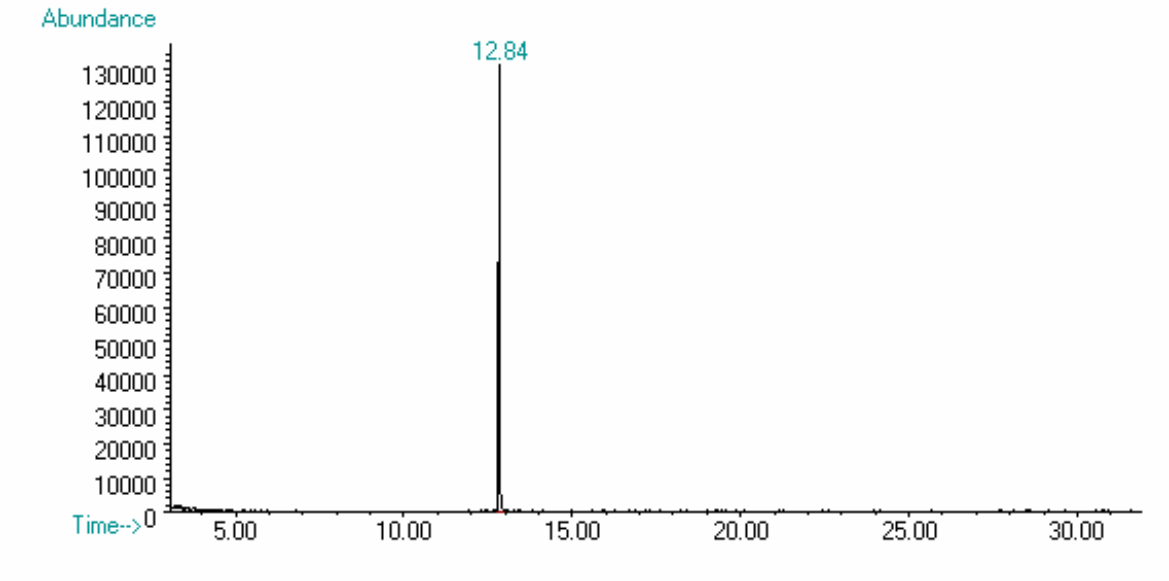


Figure B2. Gas chromatogram and mass spectra of the methyl 4-(4-methylbenzoyl) benzoate **(3)**.



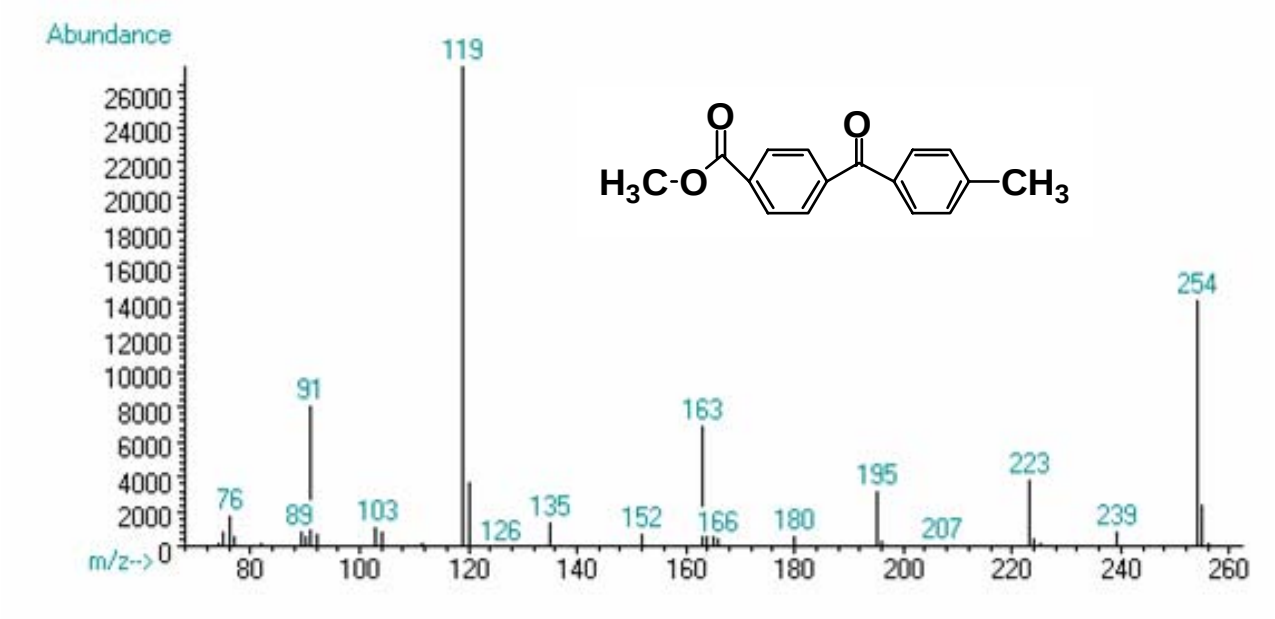
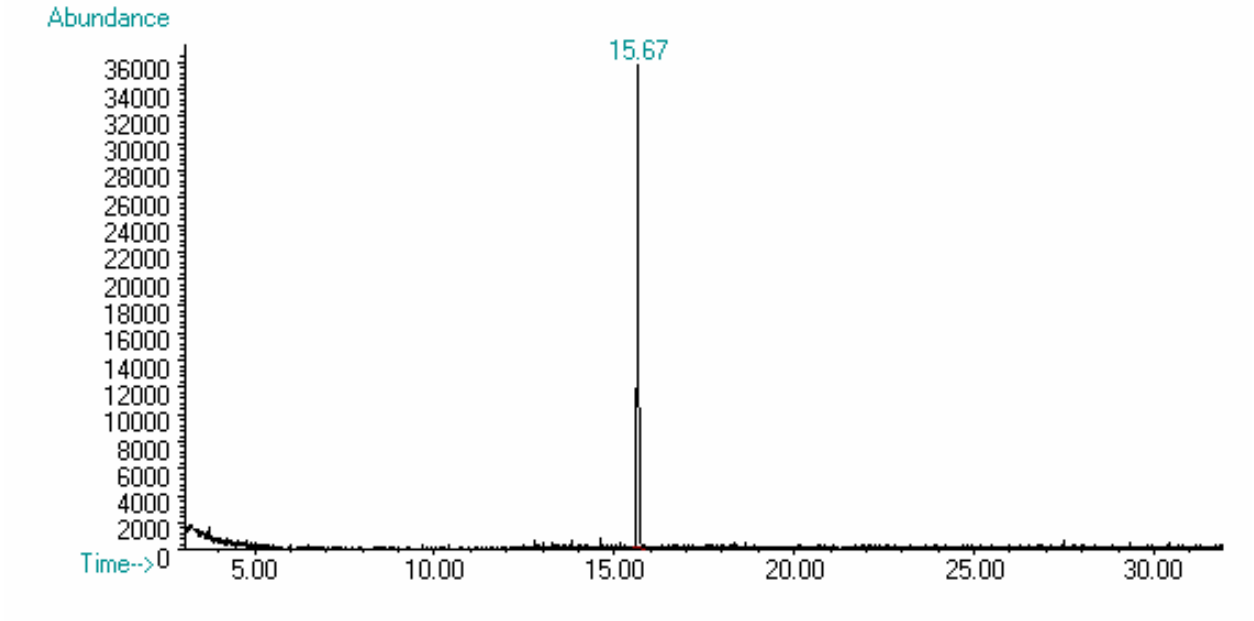


Figure B3. Gas chromatogram and mass spectra of the methyl 4-(4-romomethylbenzoyl) benzoate **(4)**.



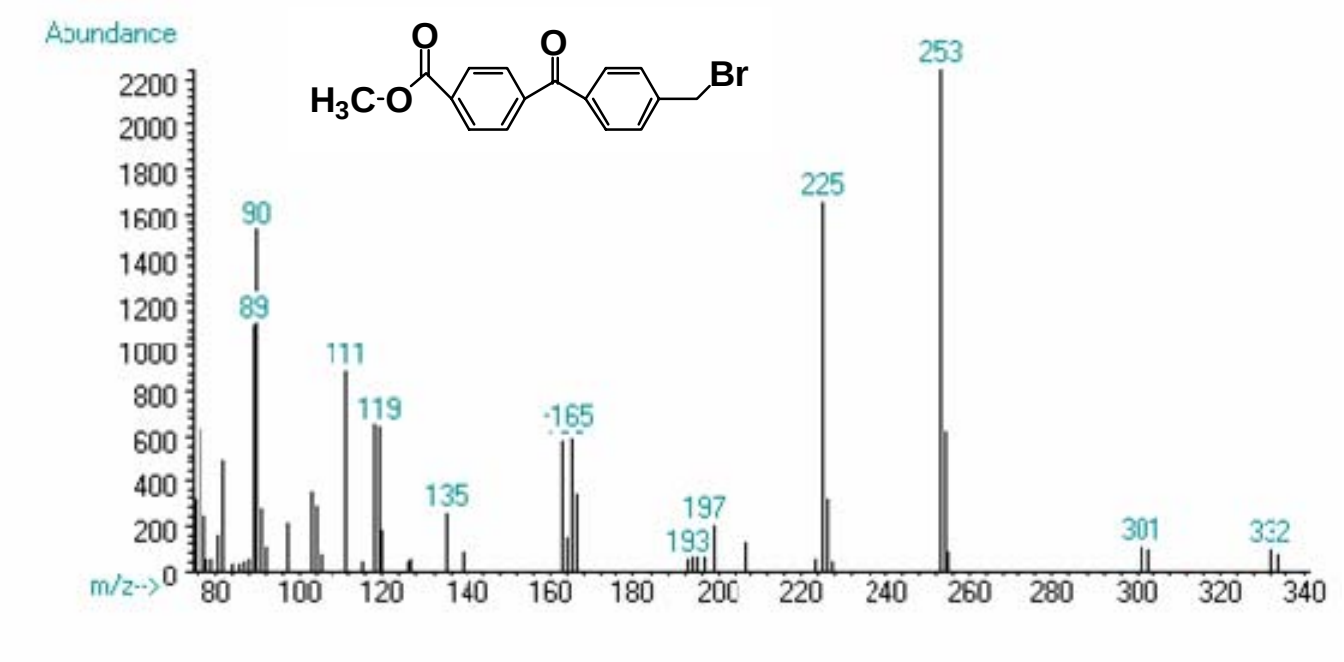
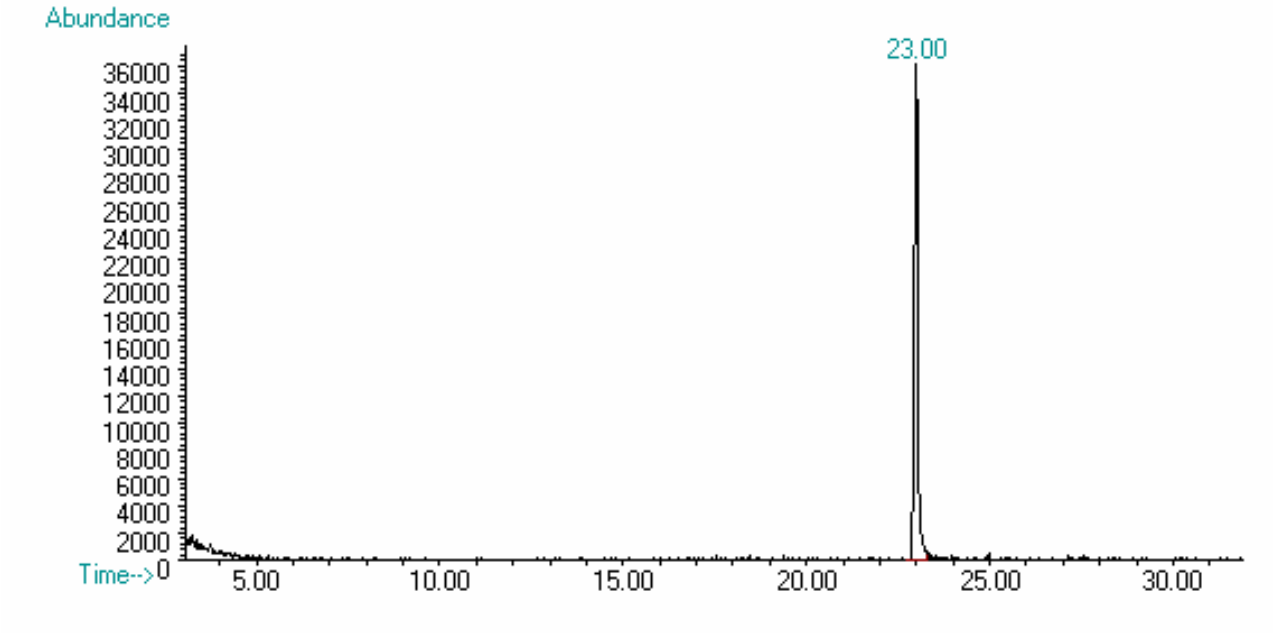
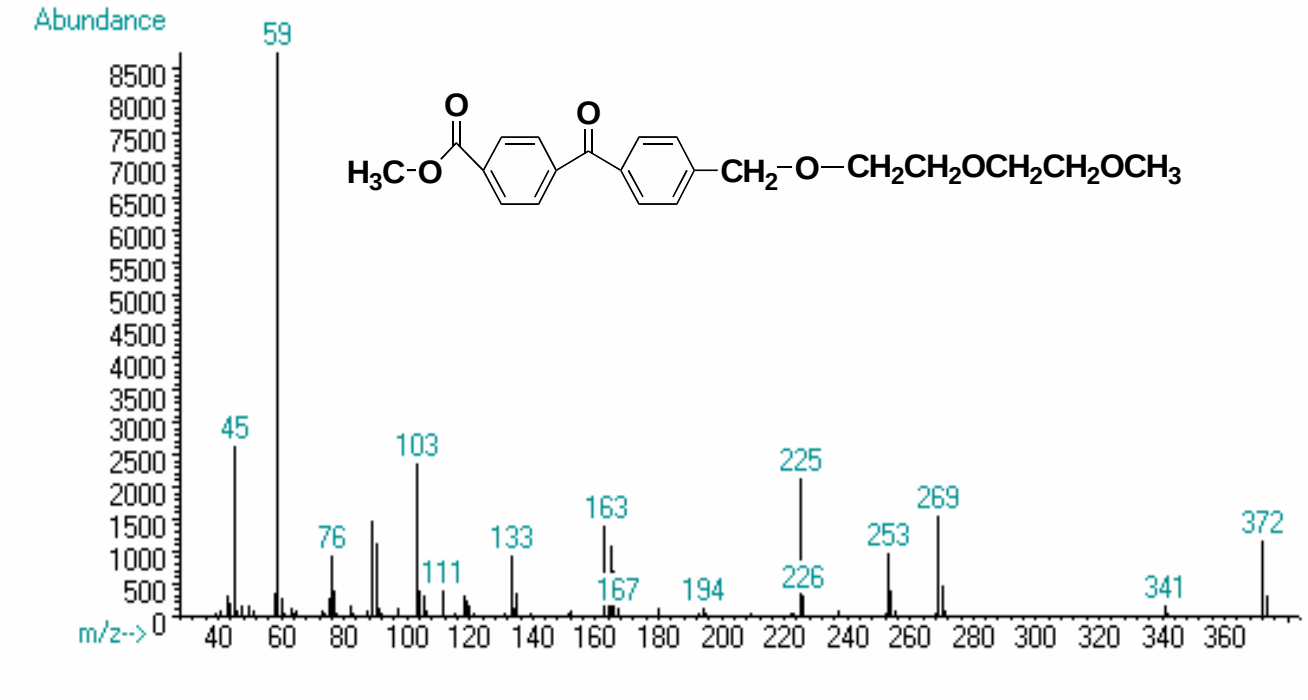


Figure B4. Gas chromatogram and mass spectra of methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoate **(5)**.





APPENDIX C

Figure C1. ^{1}H NMR spectrum of the terephthalic acid monomethyl ester chloride **(2)**.

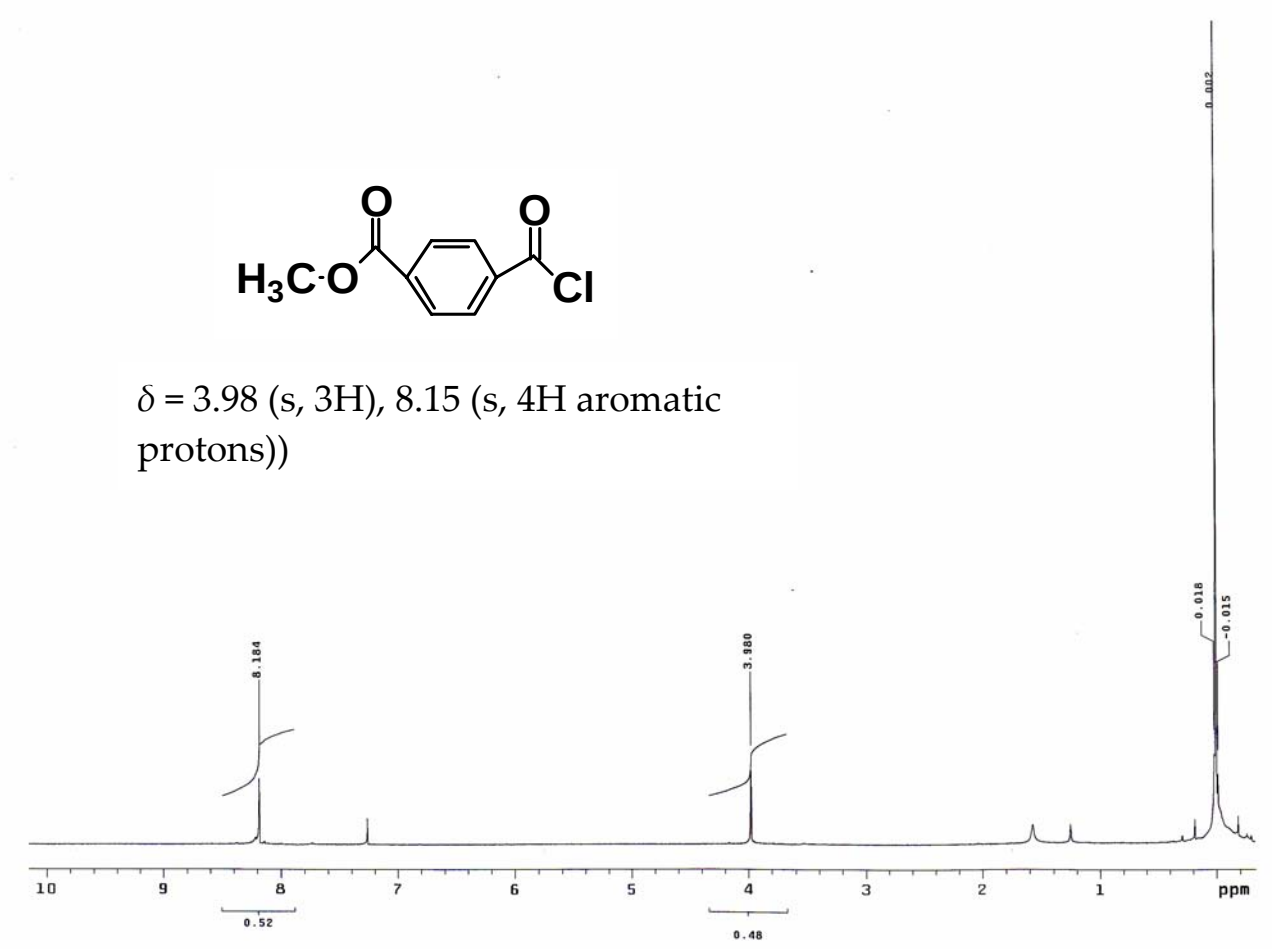


Figure C2. ^{1}H NMR spectrum of the methyl 4-(4-methylbenzoyl) benzoate **(3)**.

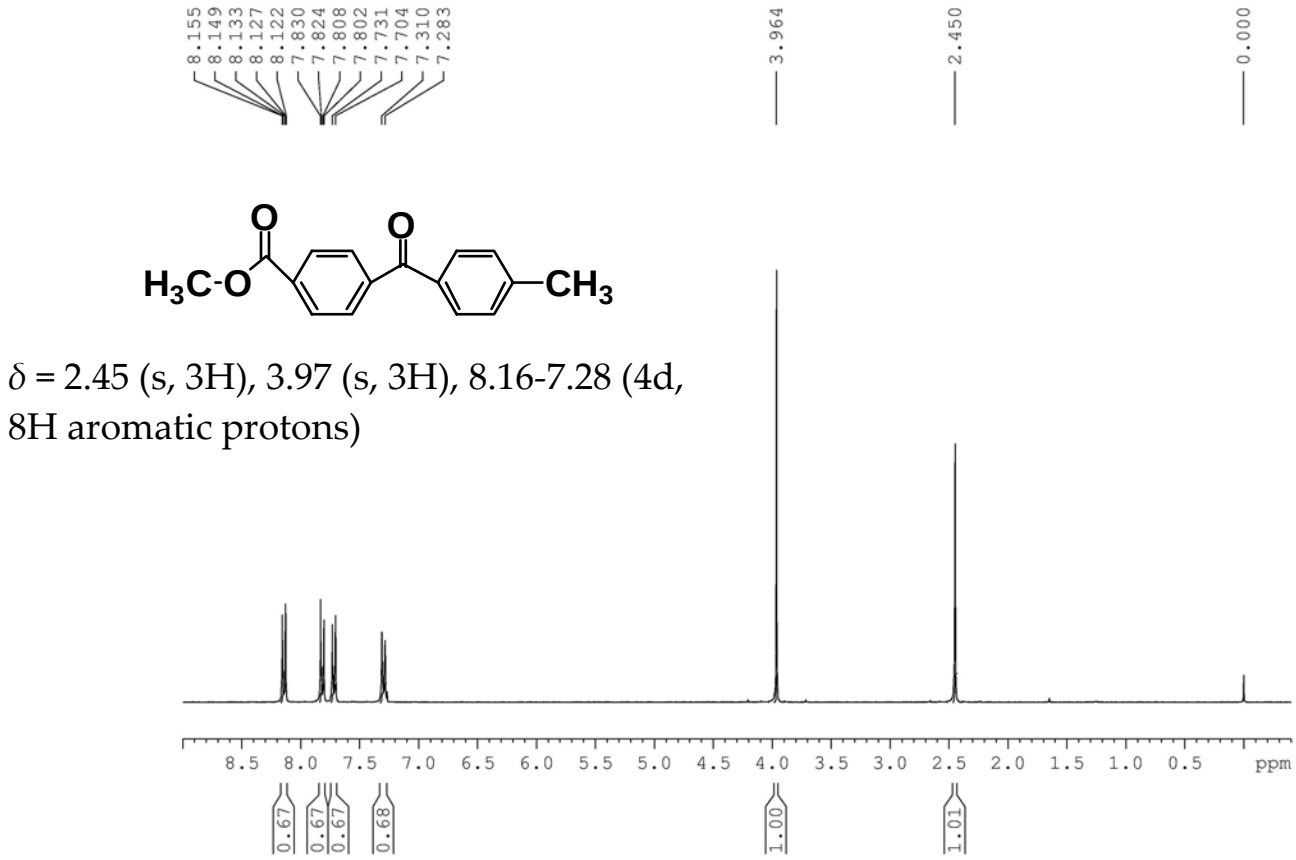
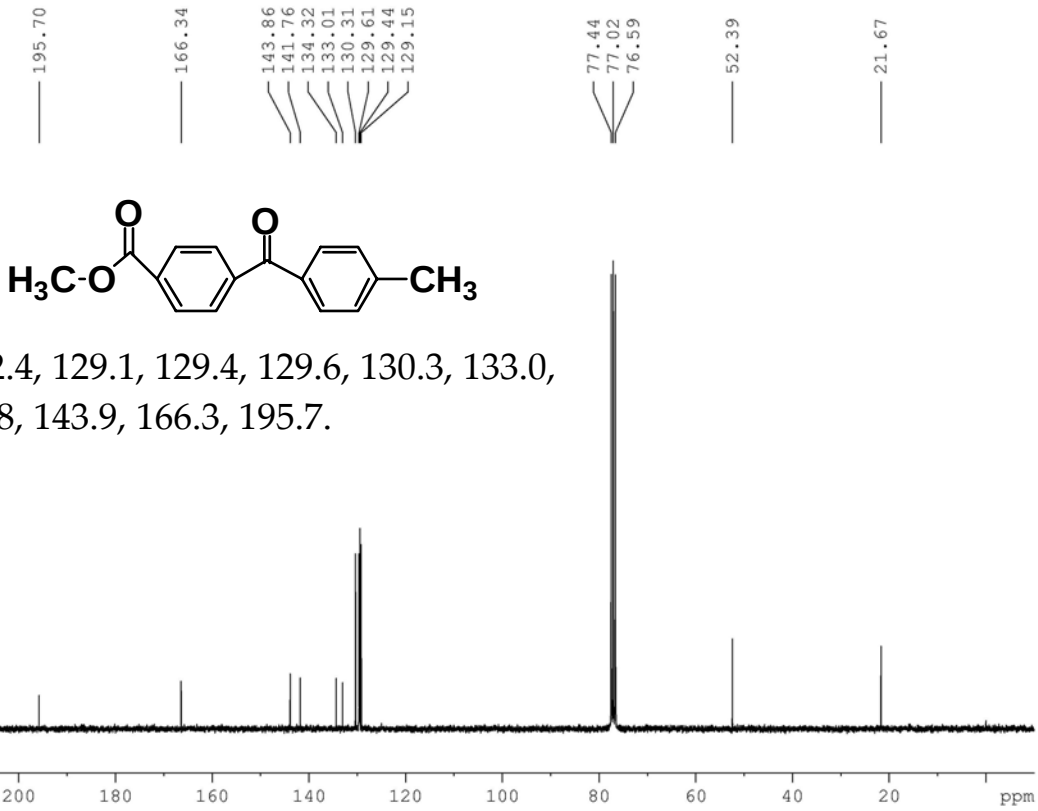


Figure C3. Decoupled ^{13}C NMR spectrum of the methyl 4-(4-methylbenzoyl) benzoate **(3)**.



δ = 21.7, 52.4, 129.1, 129.4, 129.6, 130.3, 133.0, 134.3, 141.8, 143.9, 166.3, 195.7.

Figure C4. ^{1}H NMR spectrum of the methyl 4-(4-bromomethylbenzoyl) benzoate **(4)**.

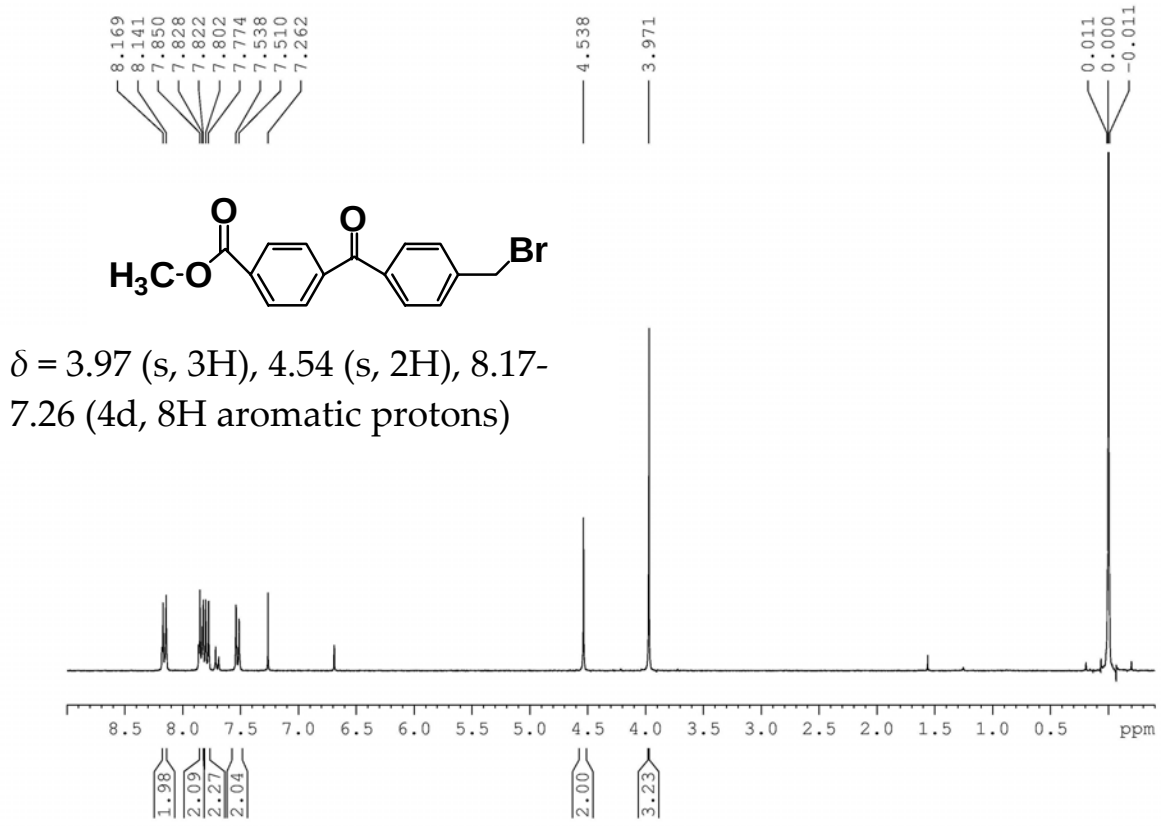


Figure C5. Decoupled ^{13}C NMR spectrum of the methyl 4-(4-bromomethylbenzoyl) benzoate **(4)**.

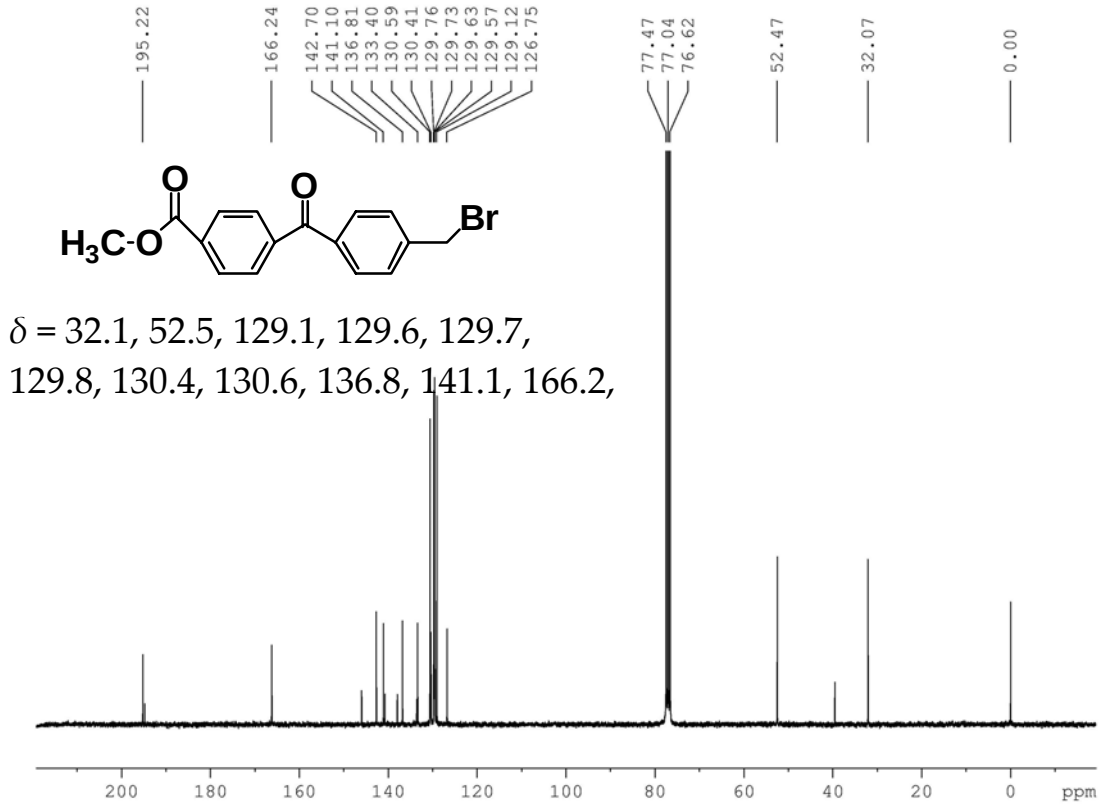
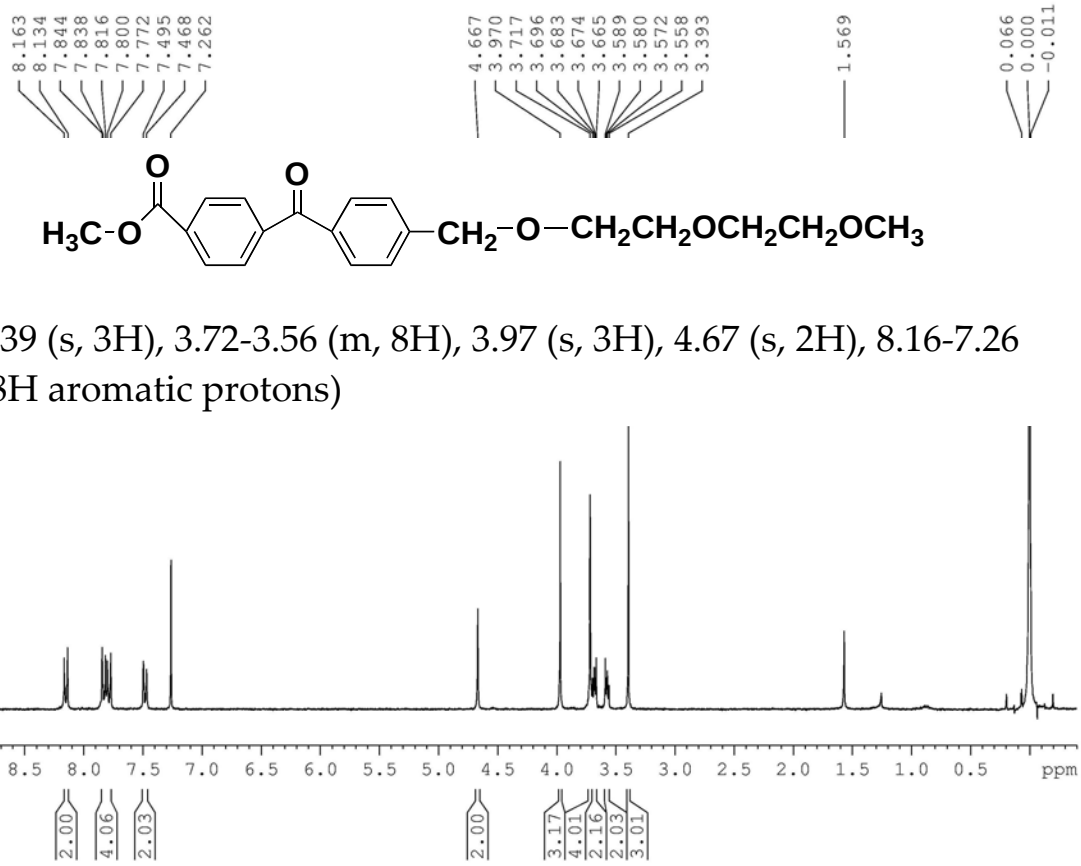


Figure C6. [1]H NMR spectrum of the methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoate **(5)**.



δ = 3.39 (s, 3H), 3.72-3.56 (m, 8H), 3.97 (s, 3H), 4.67 (s, 2H), 8.16-7.26 (4d, 8H aromatic protons)

Figure C7. [1]H NMR spectrum of the(2S)-1-Benzyloxy-2-(2-{2-[2-(2-methoxyethoxy) ethoxy]ethoxy}ethoxy)propane. Reproduced from *J. Am. Chem. Soc.* **2000,** *122*, 6175-6182 (Supporting info, page 13).

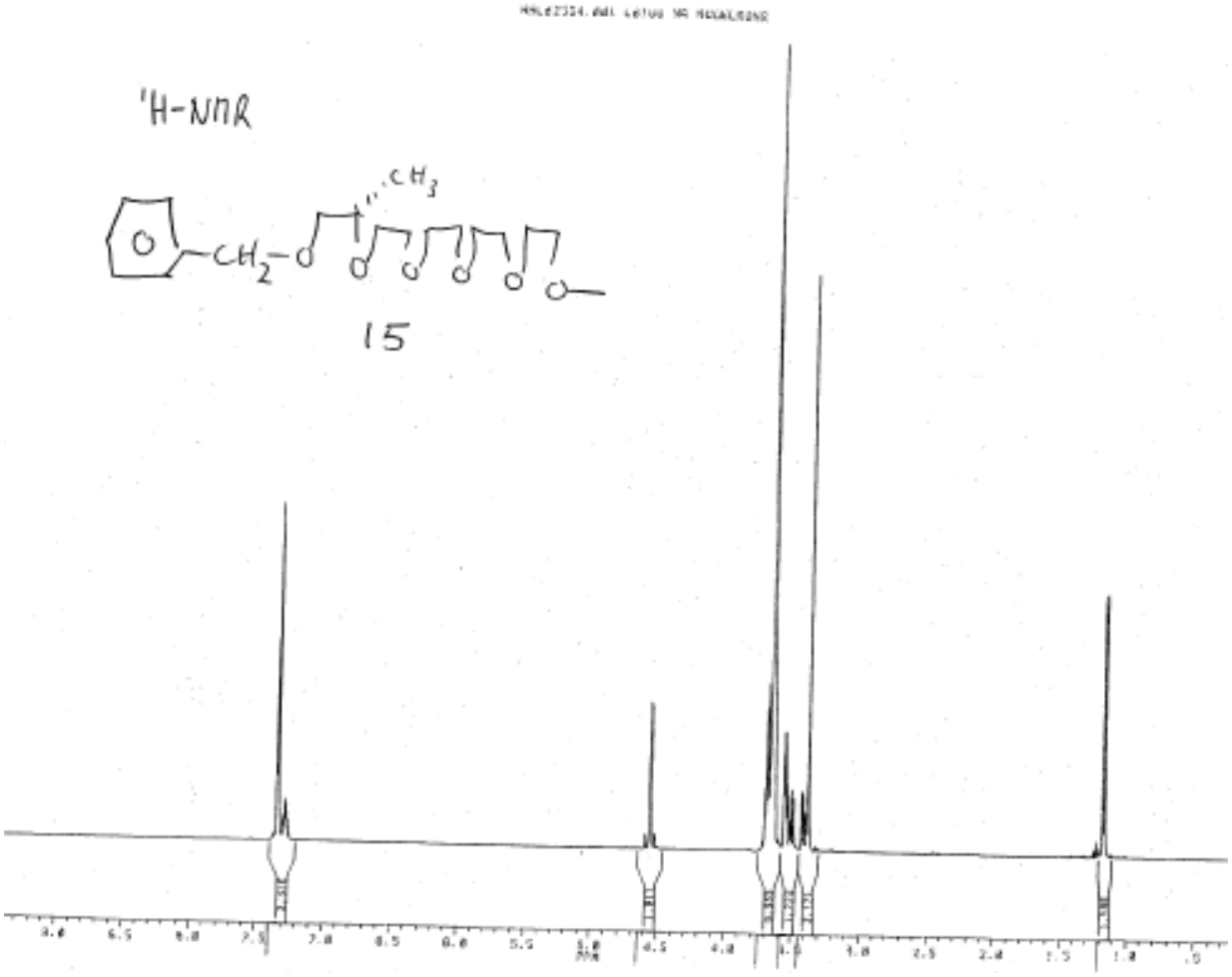
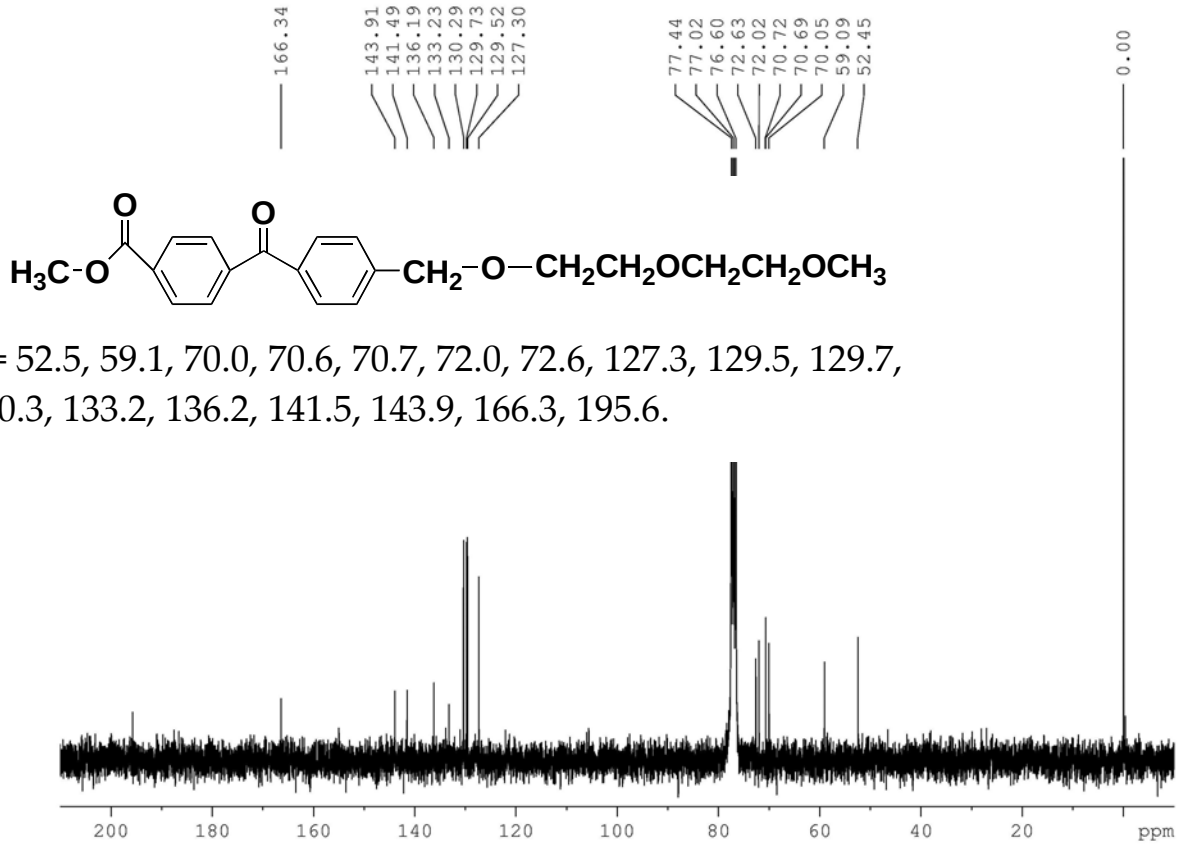
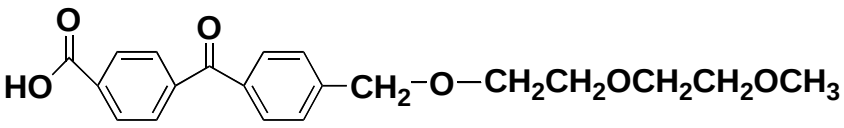


Figure C8. Decoupled ^{13}C NMR spectrum of the methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoate **(5)**.



δ = 52.5, 59.1, 70.0, 70.6, 70.7, 72.0, 72.6, 127.3, 129.5, 129.7, 130.3, 133.2, 136.2, 141.5, 143.9, 166.3, 195.6.

Figure C9. ^{1}H NMR spectrum of the methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoic acid **(6)**.



δ = 3.4 (s, 3H), 3.73-3.57 (m, 8H), 4.67 (s, 2H), 8.24-7.26 (4d, 8H aromatic protons)

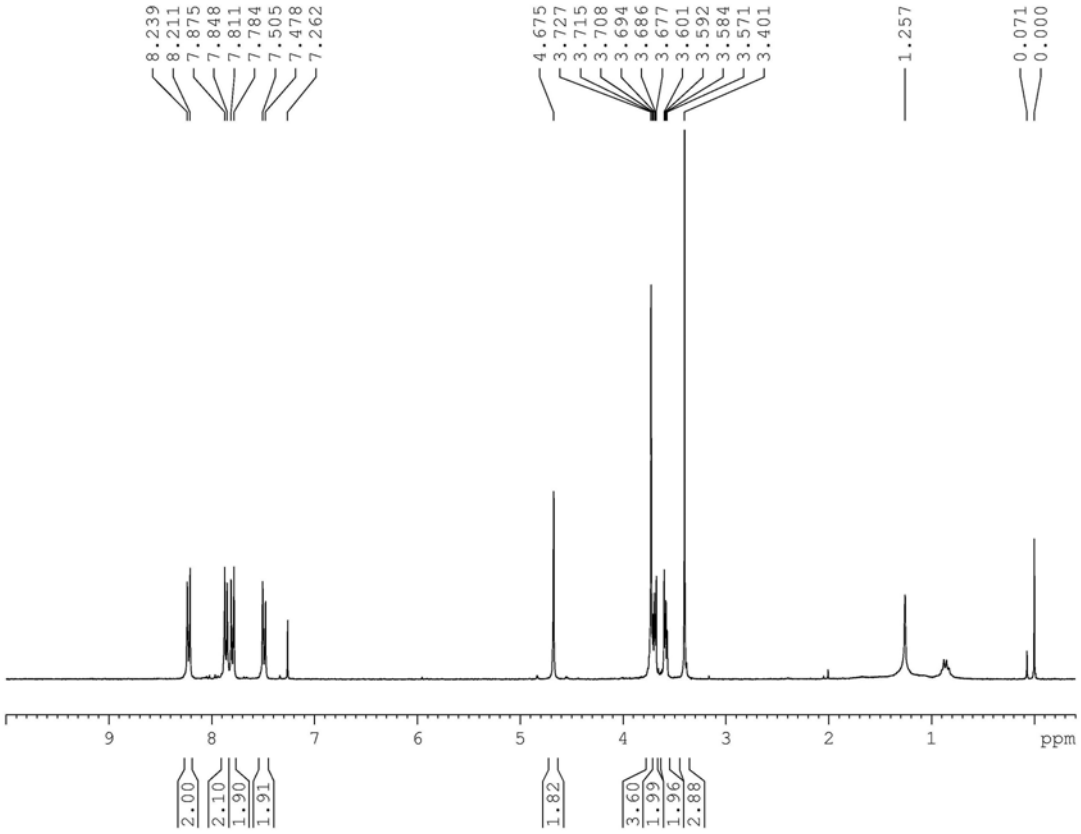
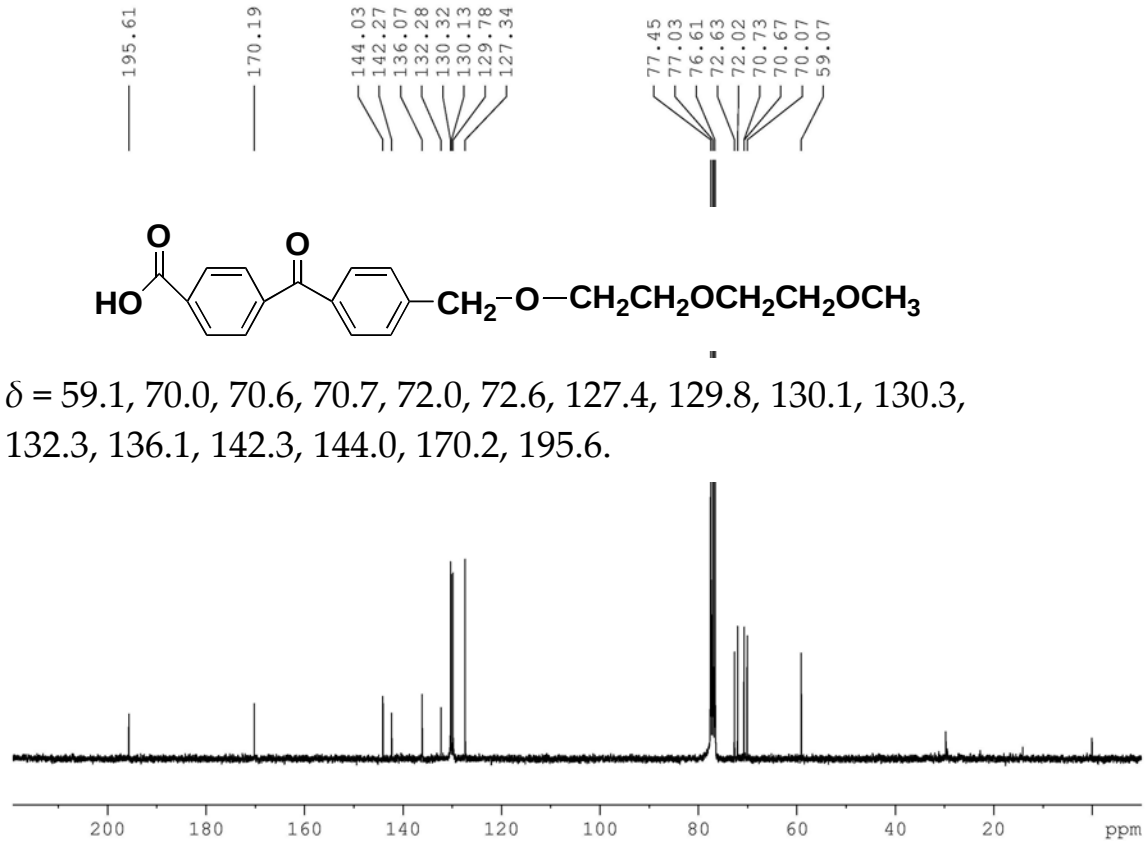


Figure C10. Decoupled ^{13}C NMR spectrum of the 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoic acid **(6)**.



δ = 59.1, 70.0, 70.6, 70.7, 72.0, 72.6, 127.4, 129.8, 130.1, 130.3, 132.3, 136.1, 142.3, 144.0, 170.2, 195.6.

Figure C11. IR spectrum of the 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoic acid **(6)**.

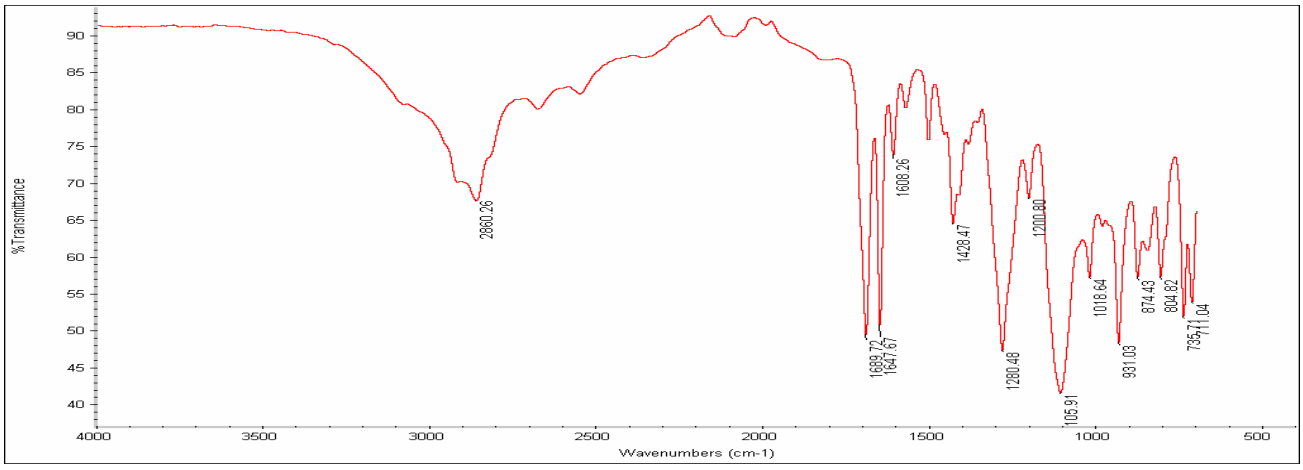


Figure C12. IR spectrum of the methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) benzoyl chloride **(7)**.

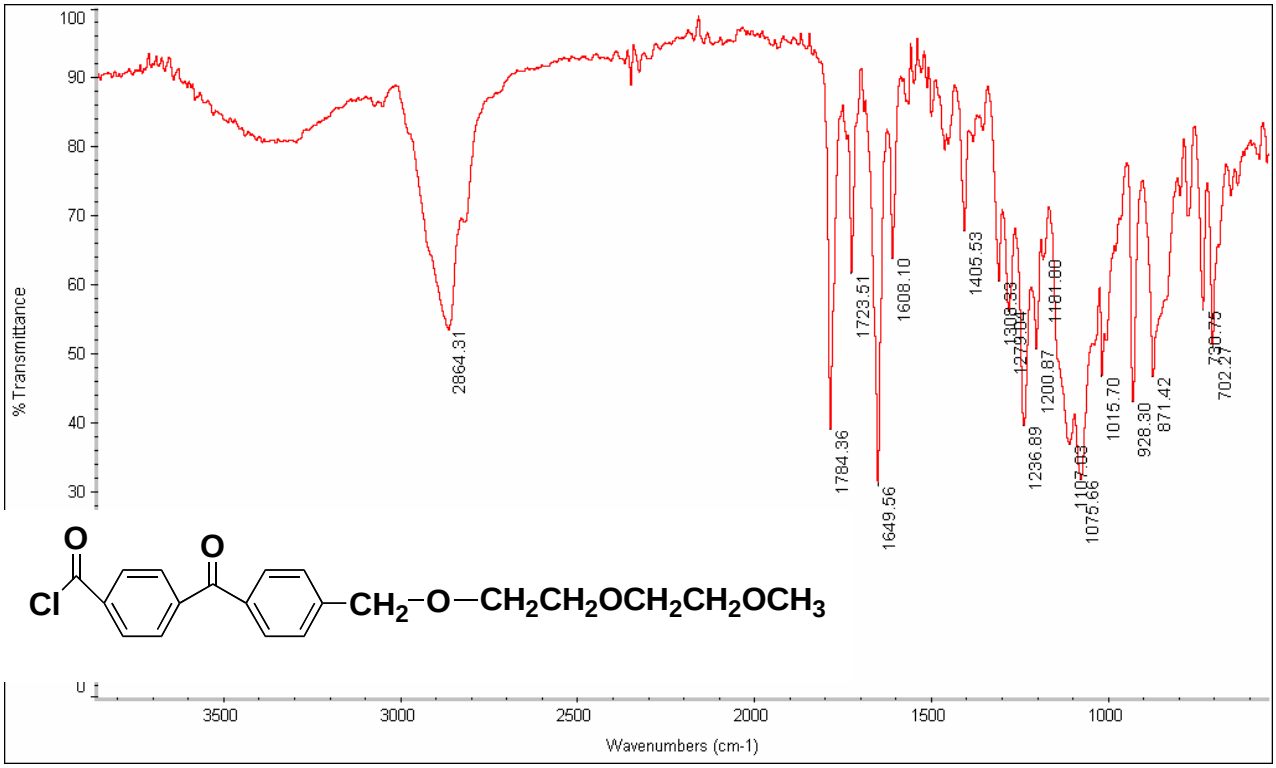
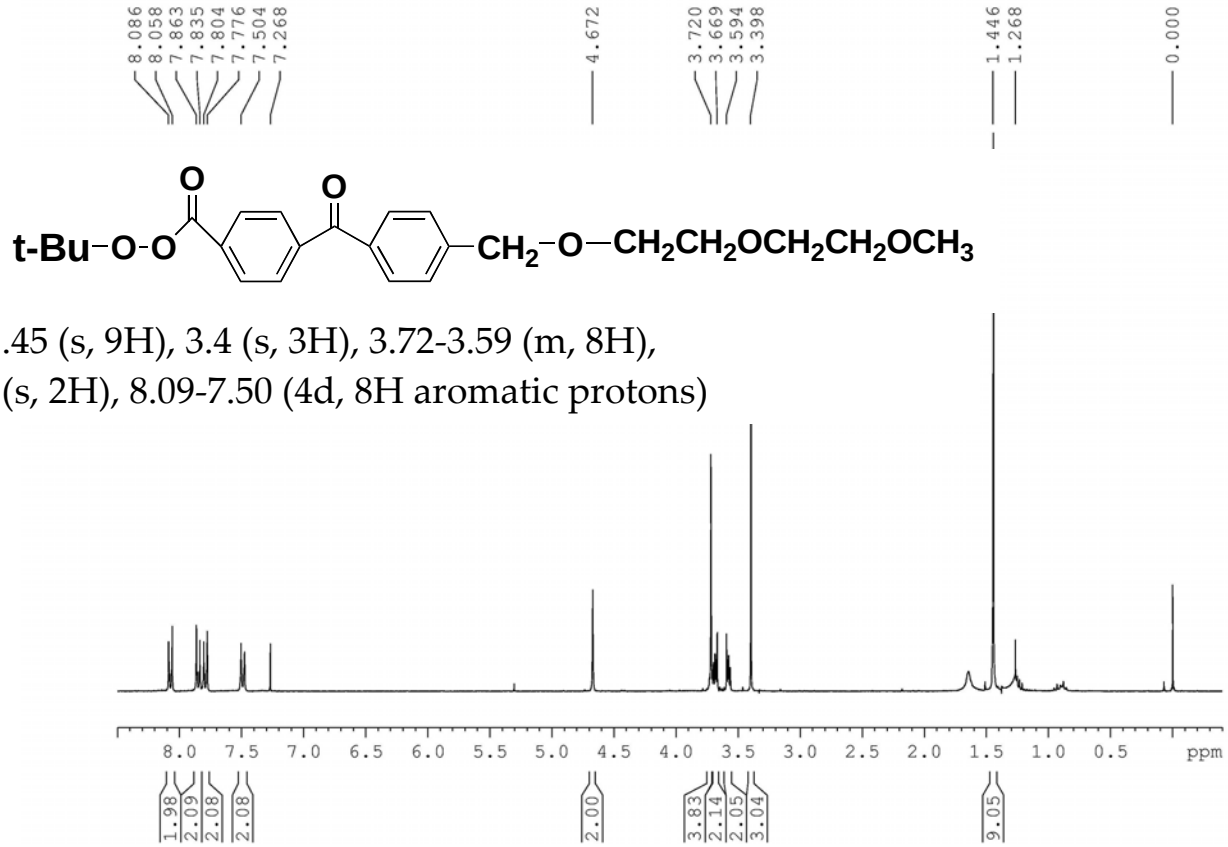
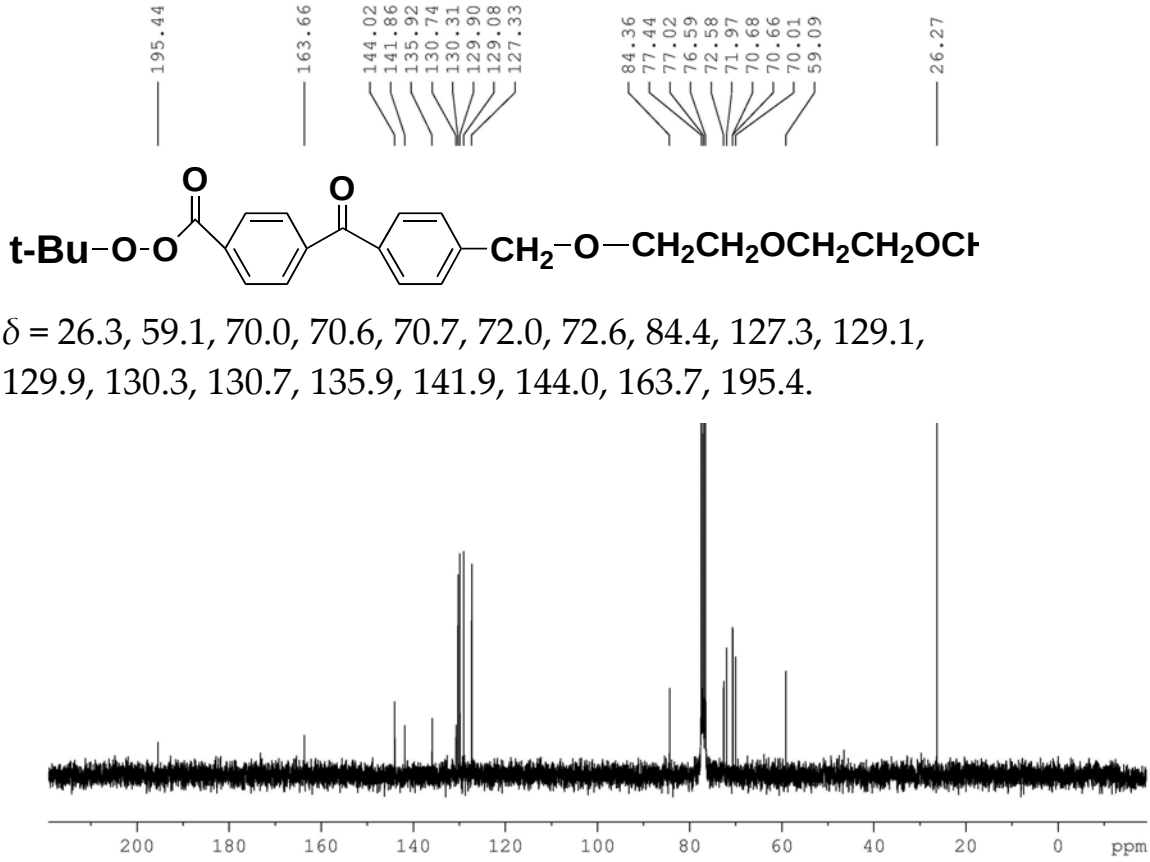


Figure C13. ^{1}H NMR spectrum of the methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) *t*-butyl perbenzoate **(8)**.



δ = 1.45 (s, 9H), 3.4 (s, 3H), 3.72-3.59 (m, 8H),
4.67 (s, 2H), 8.09-7.50 (4d, 8H aromatic protons)

Figure C14. Decoupled ^{13}C NMR spectrum of the methyl 4-(4-((2-(2-methoxy-ethoxy)-ethoxy) methylbenzoyl)) *t*-butyl perbenzoate **(8)**.



APPENDIX D

Figure D1. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with methanol

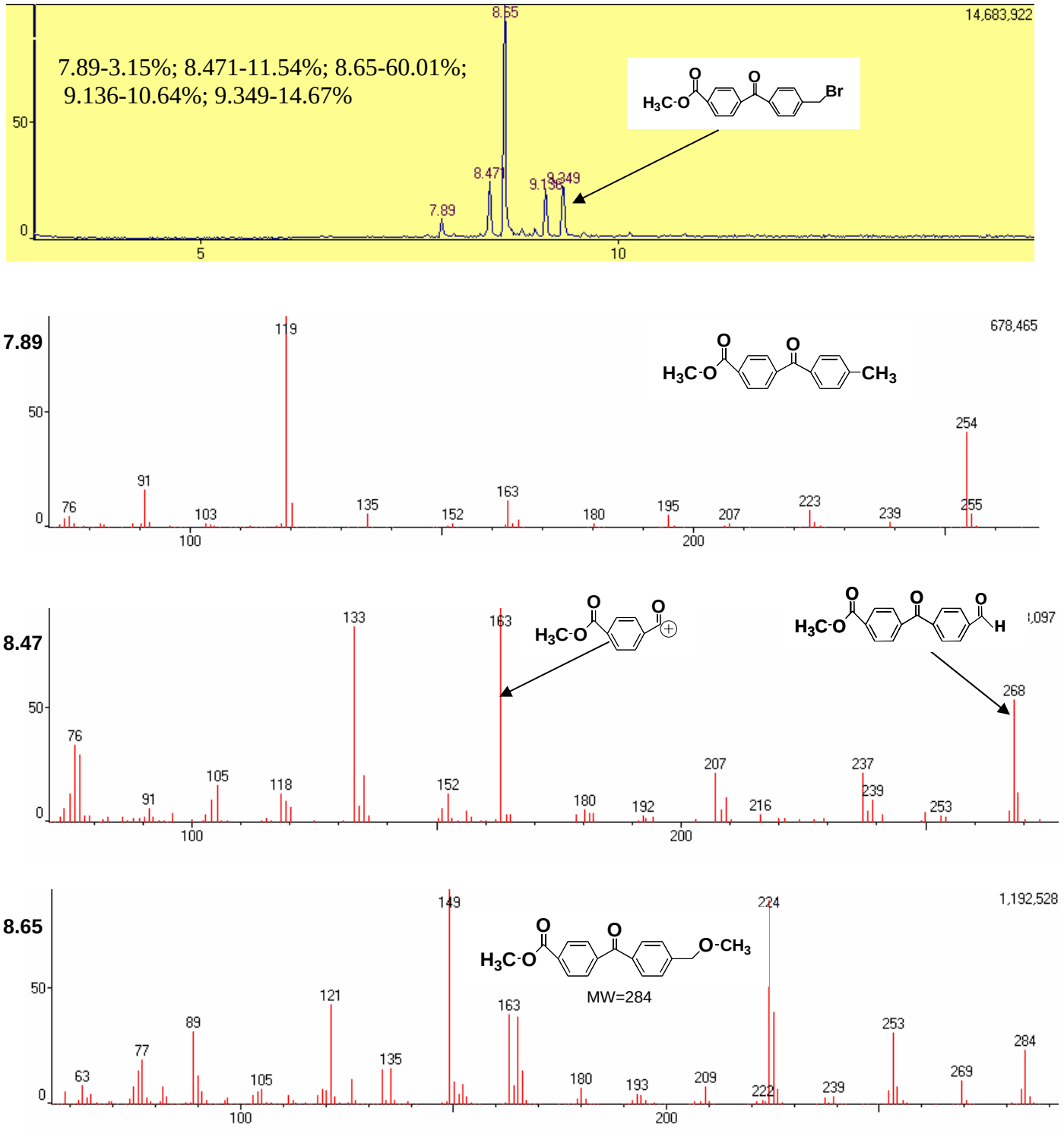
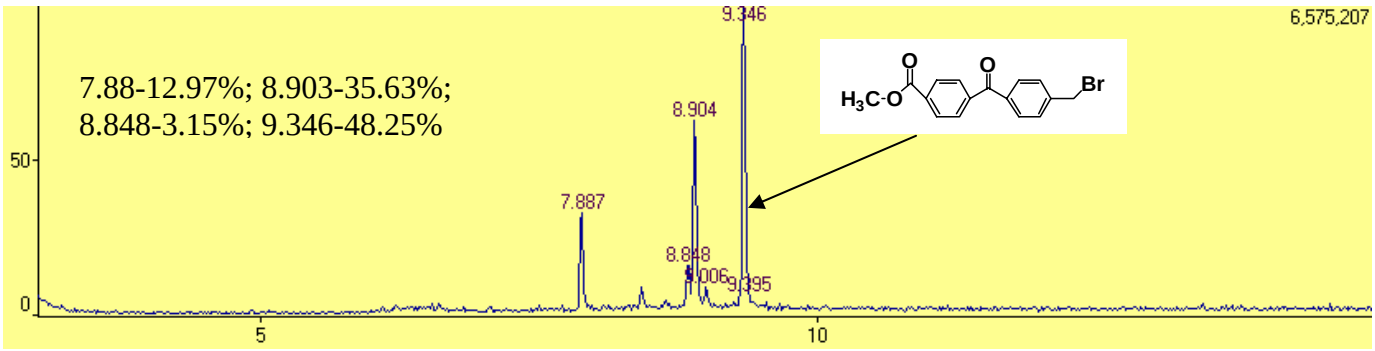
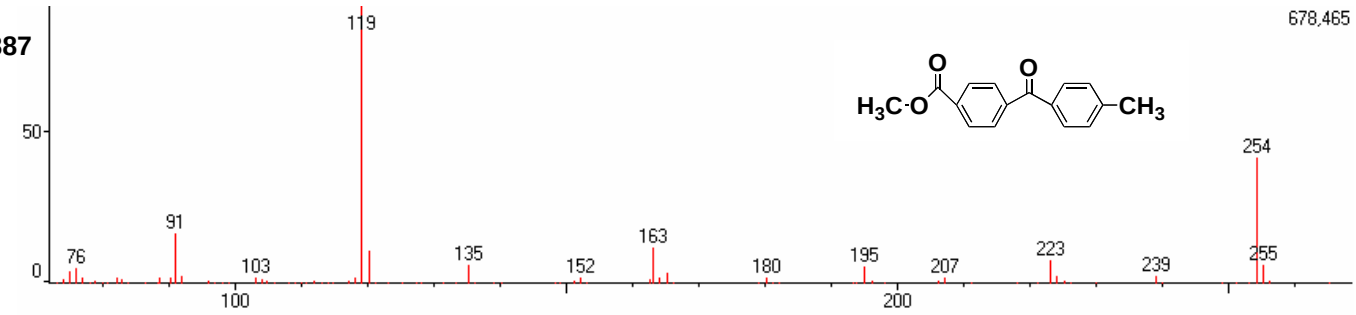


Figure D2. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with ethanol





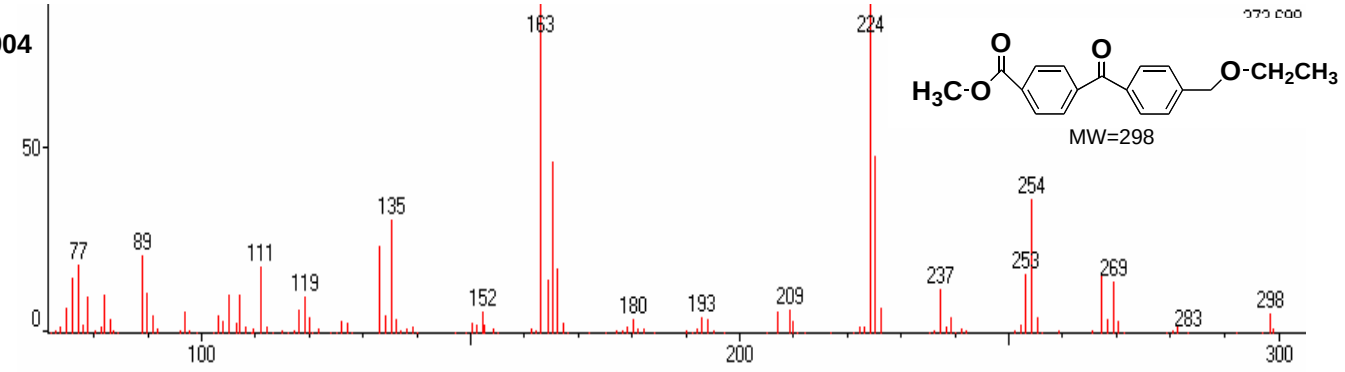


Figure D3. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with butanol

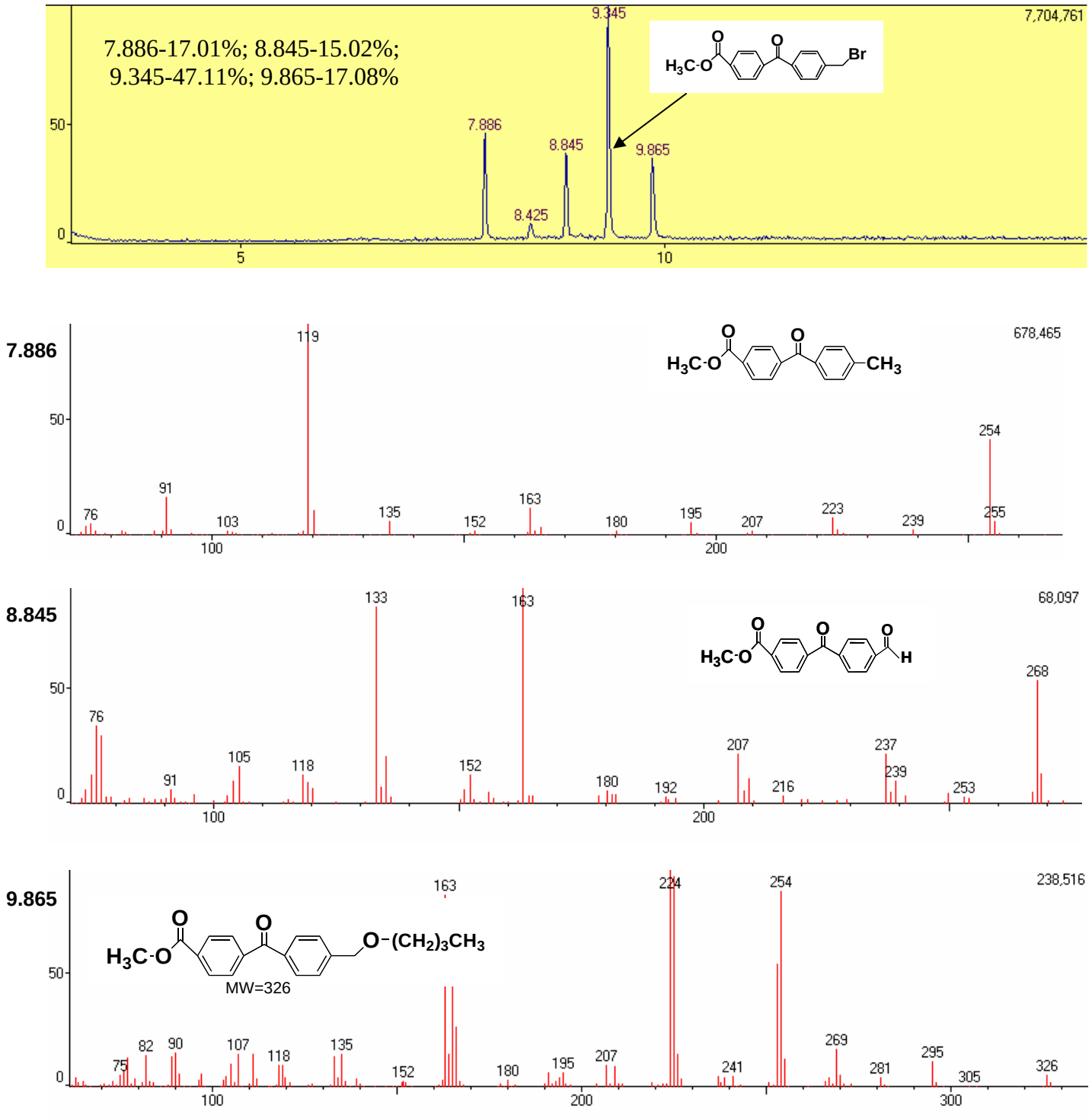
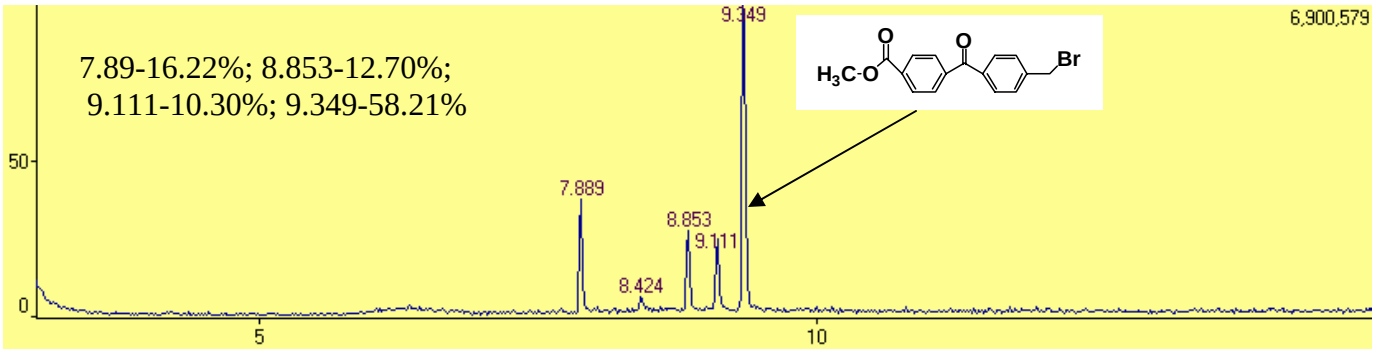
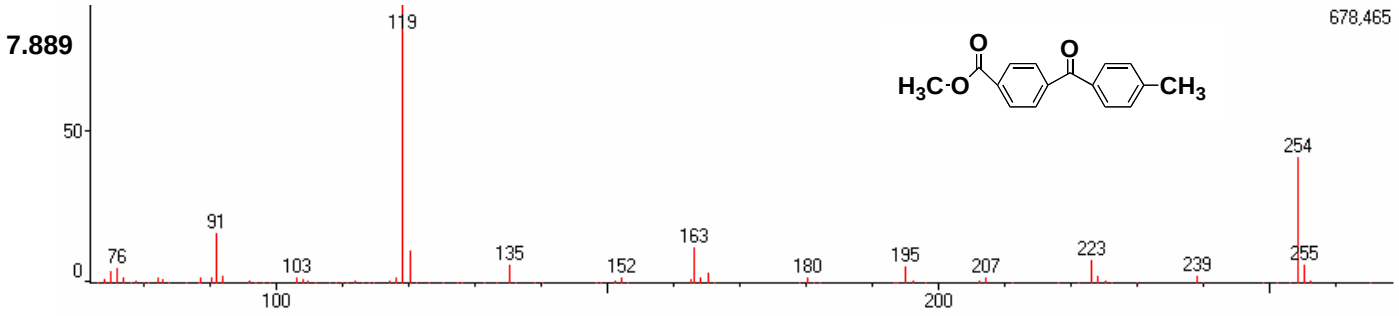
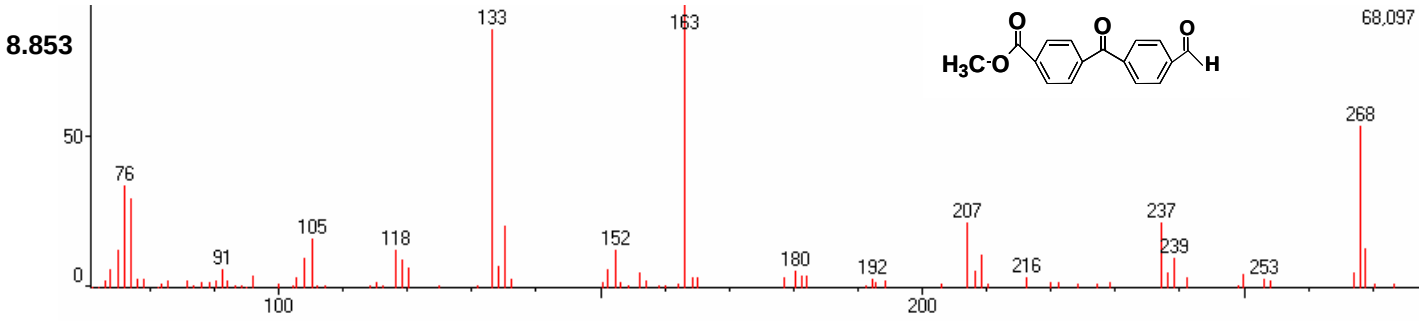


Figure D4. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with isopropanol







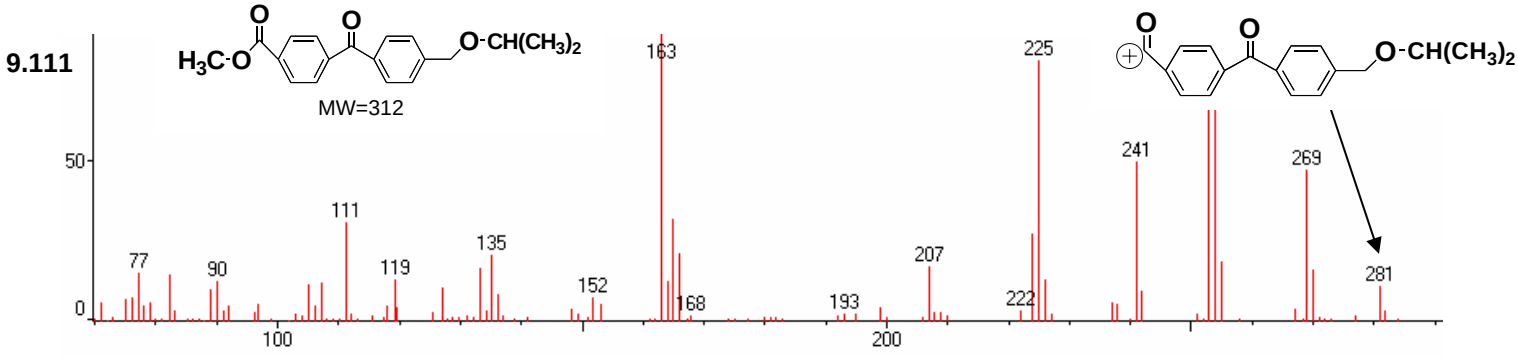
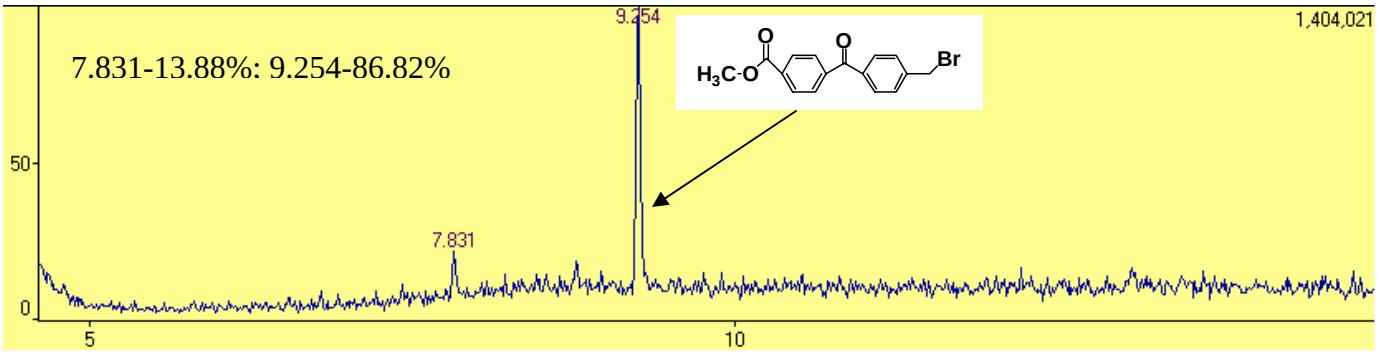


Figure D5. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with 1- octanol



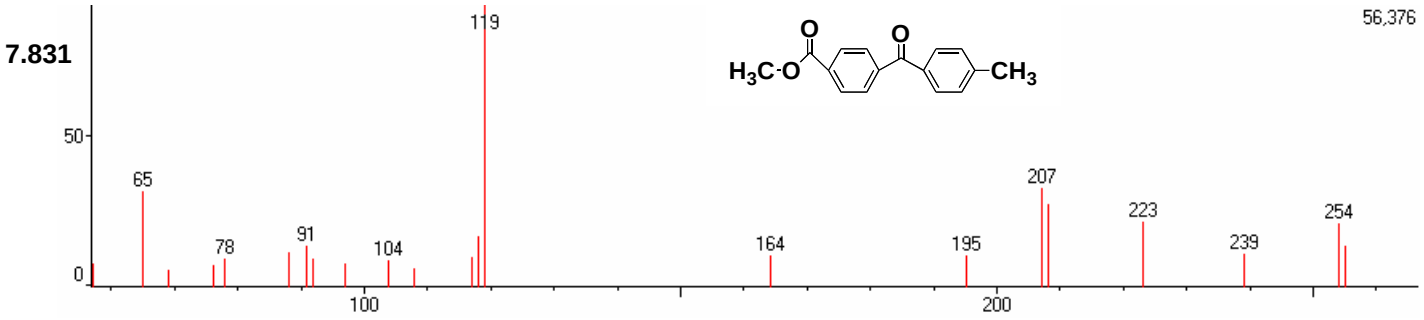
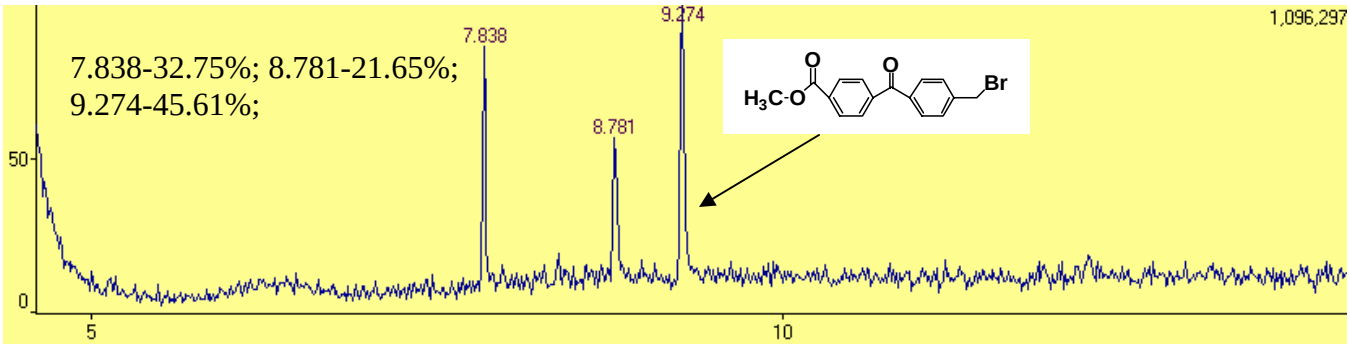
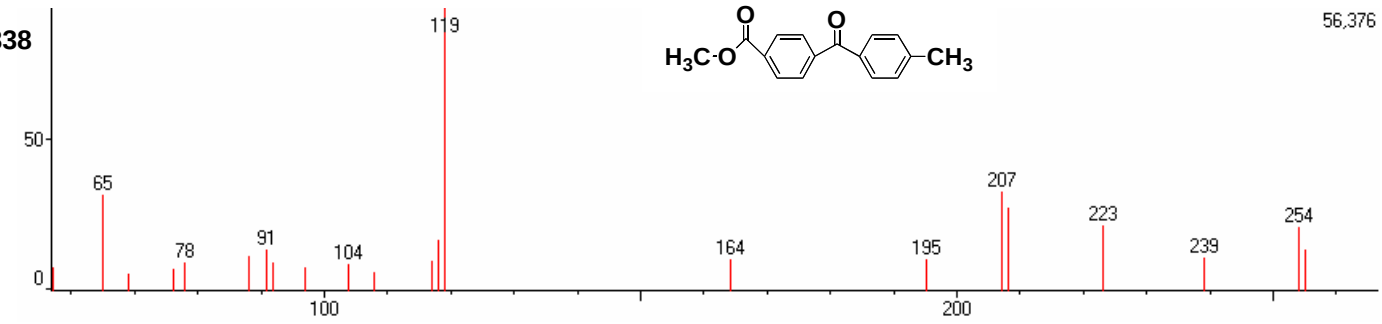


Figure D6. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with 2- octanol





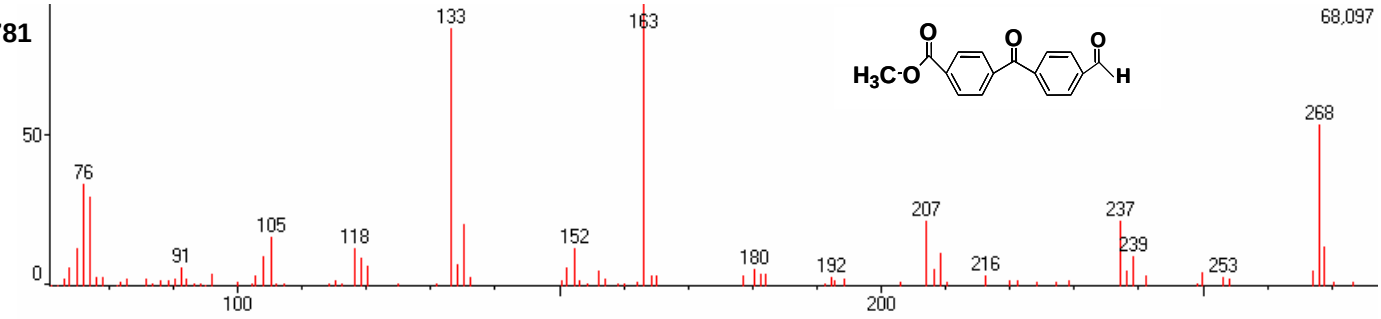
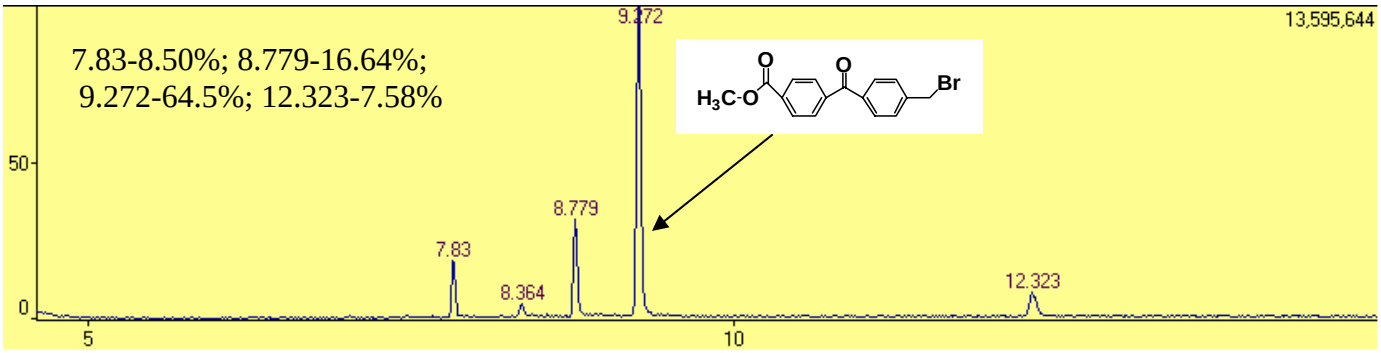
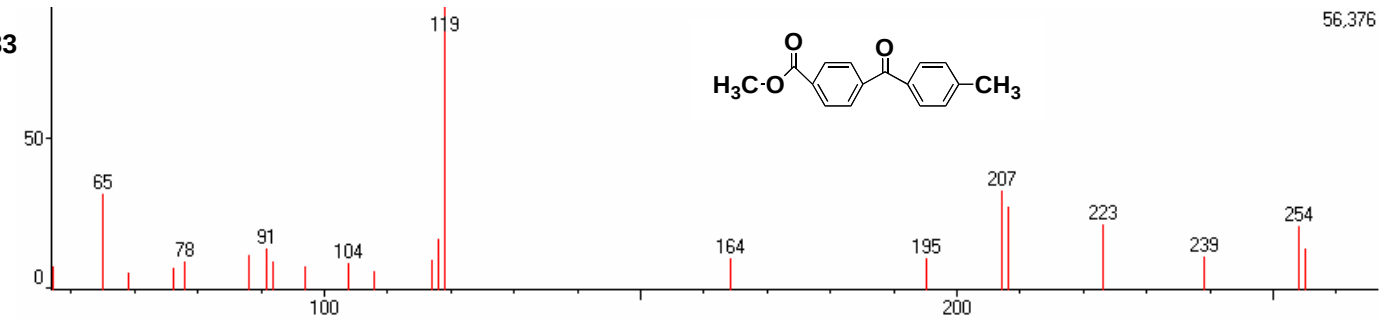


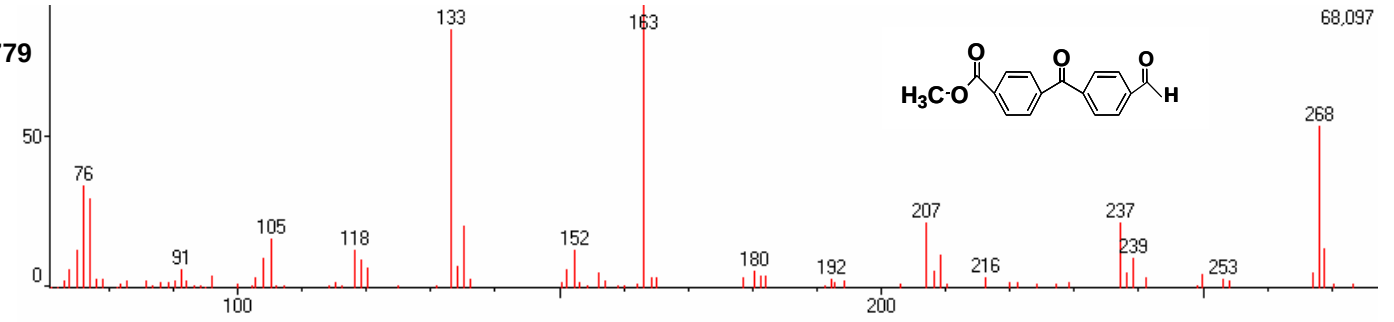
Figure D7. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with cyclohexanol



7.83



8.779



12.323

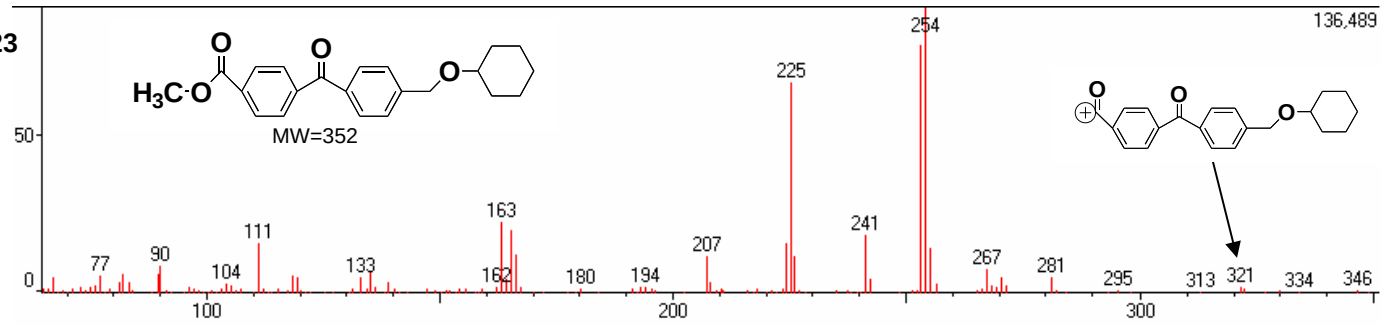


Figure D8. GC/MS of the reaction of methyl 4-(4-bromomethylbenzoyl) benzoate (4) with benzyl alcohol