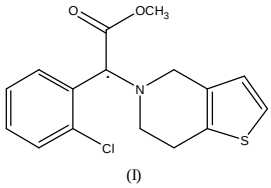
Novel salts of an anti-platelet agent

1 Introduction

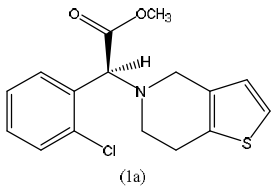
Clopidogrel, [Methyl α-5(4,5,6,7-tetrahydro(3,2-c) thienopyridyl) (2-chlorophenyl)-acetate] **(1)** is an anti-aggregatory and antithrombotic drug for the treatment and prevention of peripheral vascular, cerebrovascular, and coronary artery diseases (1-3). It was first disclosed in its racemate form in US patent number US 4529596. This document also provided a process for preparing the racemic compound which involved condensation of 4,5,6,7-tetrahydrothieno[3,2-c]-pyridine with substituted methyl 2-chloro-o-chlorophenyl acetate to provide the compound of formula (**1**) (Figure 1).



(I)

Figure 1: Methyl α-5(4,5,6,7-tetrahydro(3,2-c) thienopyridyl) (2-chlorophenyl)-acetate

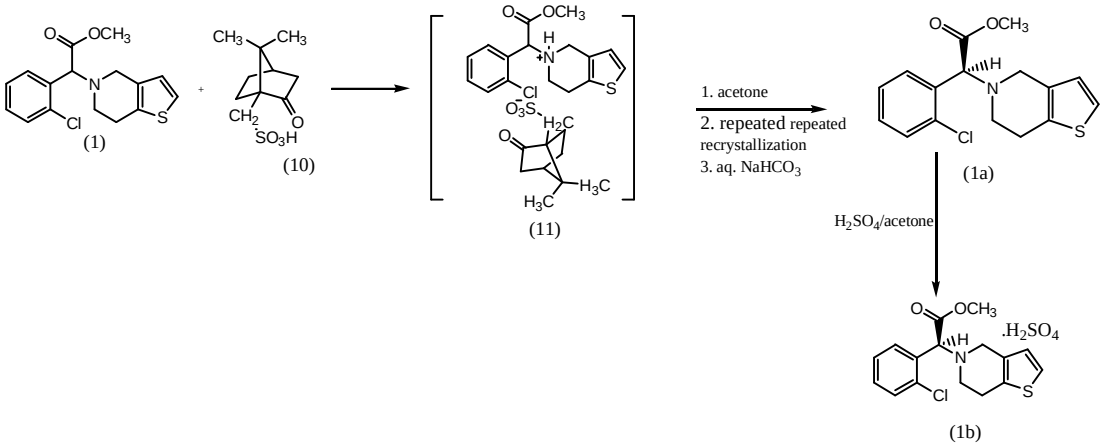
The racemate was obtained as yellow oil which was purified by converting it to its hydrochloride salt (white crystals; m.p. 130-140 °C). This compound was found to inhibit blood platelet aggregation. Subsequently, it was found that of the two optical isomers, only the dextrorotatory enantiomer (**1a**) (Figure 2) which exhibited a platelet aggregation inhibition activity, the levorotatory enantiomer being inactive. Further, it was also found that the inactive levo-rotatory isomer was less tolerated among the two enantiomers (US 4847265).



(1a)

Figure 2: methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thienopyridyl) (2-chlorophenyl)-acetate

The dextrorotatory isomer of methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thienopyridyl) (2-chlorophenyl)-acetate (**1a**) can be prepared by forming the salt of the racemic compound with an optically active acid in a solvent, repeated re-crystallization of the salt until a product of constant optical rotatory power is obtained, followed by the liberation of the dextrorotatory isomer from the salt using a suitable base. Several combinations of known resolving agents and solvent for re-crystallization have been attempted, only the levo-rotatory camphor-10-sulfonic acid in acetone gave good resolution and yields. The chiral levo-rotatory camphorsulfonic acid [(+) CSA] (**11**) was reacted with the compound of formula (**1**) in inert solvents such as acetone, dimethyl formamide, as shown below (Scheme 1):



Scheme 1: Preparation of (+)-(S)- methyl alpha-5(4,5,6,7-tetrahydro(3,2-c)thienopyridyl) (2-chlorophenyl)-acetate bisulfate salt using (+) CSA

The dextrorotatory base (**1a**, Clopidogrel base) was also obtained as oil which was purified through salt formation. Among the various salts prepared, the bisulfate salt was found to be the most promising and was taken up for development and subsequently got approved as Plavix®(**1b**).

The compound (**1a**) as its bisulfate salt [Clopidogrel bisulfate, (**1b**)] when used alone or in combination with Aspirin is known to reduce risks of cardiovascular mortality, non-fatal myocardial infraction and stroke in patients with a history of atherothrombotic diseases (4). The compound (**1a**) is an inactive pro-drug that gets converted to the pharmacologically active metabolite *in vivo* through the hepatic metabolism to exhibit its anti-platelet effect (5). It is first converted by the action of cytochrome P450 (P450) to the corresponding 2-oxo derivative (a thiolactone), then in a second step it is converted to the pharmacologically active, thiol-containing metabolite (6) (Figure 3). The P450 isoforms involved in the bioactivation of the compound (**1a**) have been suggested to be CYP1A2 in rats (7) and CYP3A in humans (8), although the contribution of these P450s to produce the active metabolite is still unclear. In addition, several recent clinical studies demonstrated that CYP3A4, CYP3A5, and CYP2C19 have a significant role in the formation of the active metabolite from (**1a**) (9-13).

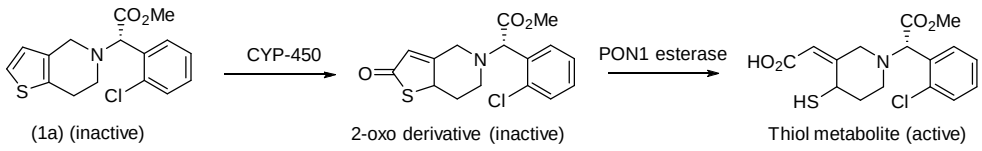


Figure 3: Metabolic pathway of compound (1a)

The free base form (**1a**) is unstable owing to a labile proton at the chiral center and susceptible to racemization (1). As described earlier, the racemic form of the base is less active and it is only the (s)-isomer which is therapeutically effective. Therefore, the free base (**1a**) must be stabilized by salt formation through use of suitable acids. It is commercially available as the bisulfate salt. The bisulfate salt was first disclosed in US patent no. 4847265. Several polymorphic forms of the bisulfate salt are already described in the literature, of which the commercially marketed polymorphic forms are commonly referred to as Form 1 & Form 2. The drug was initially launched in the polymorphic Form 1 but later, switched to the polymorphic Form 2 due to the greater stability and better pharmaceutical properties of the Form 2. Nowadays, it is available commercially in the polymorphic Form

2. Being a valuable pharmaceutical substance, its synthesis is protected by several patents. Several alternate routes of synthesis of compound (**1a**) have been reported in the recent literature including those involving the use of catalytic asymmetric Strecker reaction (15), Mannich-like multi-component synthesis (16); biosynthesis of certain key intermediates used in the synthesis of the compound have also been reported (17-18).

2 Chemistry & Rationale:

(S)-o-chlorophenyl glycine is an important intermediate in the preparation of compound (**1a**). Several processes have been reported for the preparation of this key intermediate and its conversion to the open chain compound **8** (19-23). However, many of these processes use chemicals, some of which are strongly lachrymatory and mucous membrane irritants (24, 25). Use of such chemicals poses difficulties during handling and up-stream processing; further these chemicals are also unfavorable for human health and environment. Therefore, in one aspect, an improved process for preparing the compound (**1a**) has been designed involving intra-molecular cyclization of the compound **8** using 1,3-dioxalane and catalytic amount of protic acid to obtain the compound of formula (**1**) in its racemic form which was then separated to its enantiomeric forms using levo-rotatory camphor sulfonic acid [(+) CSA] (Scheme 2).

The polymorphic Form 2 of the bisulfate salt of compound (1a), claimed in US 6429210, is patent protected till atleast 2018. Although a valuable therapy, non-responsiveness or poor responsiveness to therapy with the compound (**1b**) is widely prevalent leading to higher risk of death, myocardial infraction and stroke (26). Also, a major drawback of the bisulfate salt is that there is an increase in the amount of the inactive metabolite, (+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]-pyridine-5(4H)-acetic acid (27, 28) over the six month period. All of these may sometimes lead to variability in the effectiveness in different bisulfate preparations available commercially (29, 30). Therefore, there exists a need to prepare new salts of compound of formula (**1a**) which may provide certain

advantages over the bisulfate salt. US 4847265 states that attempts to make several acid addition salts of (**1a**) were not successful and the salts could not be isolated in pure crystalline form. Certain solvated forms of the benzene sulfonate salt of (**1a**) have been reported in EP 1480985 (31) (the toluene and dioxane solvates). Other organic salts of the compound of formula (**1a**) have also been reported (32-35). However, none of these salts are reported to have any advantages over the bisulfate salt and does not appear to overcome the problems with the bisulfate salt discussed above. Another aim of the present work is therefore to solve some of these problems through the formation of alternate salts of the (**1a**). Preparation of alternate salts is the only viable route because the base is an oil (US 4847265). Attempts to crystallize the base were unsuccessful and therefore, making alternate polymorphic form of the base was not feasible. Inorganic acid salts of the compound (**1a**) were already reported and the HCl and HNO_3 are expected to have similar problems as the bisulfate salt as each of them are strong acids. Therefore, attempts have been made to prepare organic acid salts of compound (**1a**), preferably with weak organic acids.

Crystalline solids are generally preferred over the amorphous forms for the following reasons among others:

- They are easier to purify (for example by recrystallization);
- can have a defined purity required for the approval of a pharmaceutical;
- is readily detectable and identifiable by customary methods such as XRPD, (X-ray powder diffraction), Raman spectrum, IR (infrared spectrum), and it has a reproducible physical quality;
- a better chemical stability during storage;
- is generally less hygroscopic than the amorphous form.

Also, crystalline active ingredients are generally more stable than corresponding amorphous active ingredients. Problems with the degradation of the active ingredients can also be avoided.

The amorphous form of an active ingredient may also comprise unwanted quantity of solvents. These are generally difficult to remove, since re-crystallization is not possible.

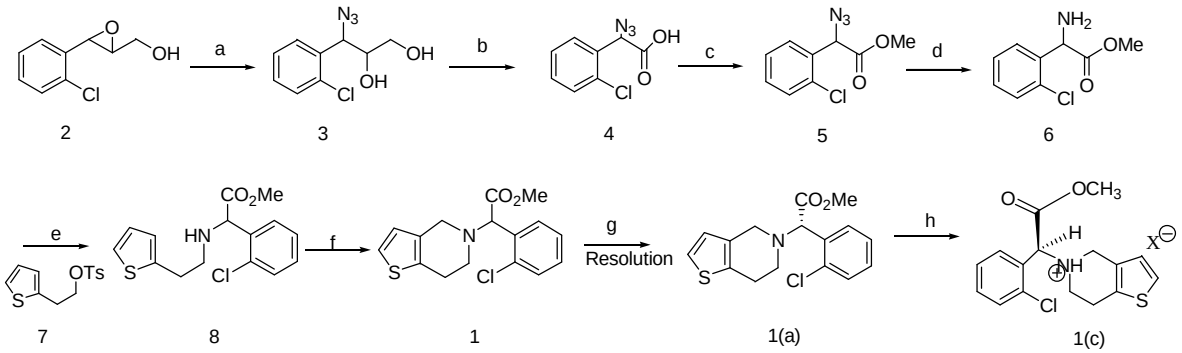
The amorphous form contains more energy than the crystalline form. This may lead to the random pattern of the distribution of the molecules of the amorphous form rearranging spontaneously with release of energy, and partial dissipation of energy. This may result in changes in the activity of the active ingredient without this being directly evident in a measurable parameter of the active ingredient. The consequence is a significant effect on the reliability of the active ingredient and thus a risk for the patient. Also, it is difficult to prove that different batches of the amorphous active ingredient are identical.

Because of these and other reasons including pharmaceutical processability etc. crystalline salts of the compound (**1a**) are preferred. Therefore, the aim was to prepare organic acid salts of compound (**1a**) in crystalline form.

2.1 Chemistry

The compound of formula (**1a**) and its organic salts were synthesized as described in Scheme 2. 2-chloro epoxy cinnamyl alcohol **2** was heated with sodium azide and ammonium bromide in a mixture of ethanol and water to yield the azido diol derivative **3** (36-38). Oxidation of **3** using sodium metaperiodate and catalytic amount of $RuCl_3$ in a mixture of acetonitrile, water and carbon tetrachloride gave **4** (36, 39, 40) which was subsequently esterified by treating with thionyl chloride in methanol to give azido ester **5**. Reduction of azide group under catalytic hydrogenation conditions using palladium on charcoal as catalyst gave the intermediate **6**. Coupling of **6** with **7** in presence of sodium bicarbonate in dimethyl formamide gave intermediate **8**. The preparation of **8** as described herein is as per processes known in the literature and the characterization data for all the intermediates matched with those reported and have not been reproduced. Heating of **8** in 1,3-dioxalane and methanolic HCl at 70 °C resulted in cyclization to give the racemic compound **1** (41) in quantitative yield.

Resolution of **1** using (1S)-(+)-camphor-10-sulfonic acid yielded the dextro-rotatory isomer **(1a)** which was subsequently converted to its organic salts **(1c)** by the treatment with appropriate acids in suitable solvents.



Scheme 2: Reagents and conditions: (a) NaN_3, NH_4Br, ethanol and water, 75 °C, 2 hours (b) sodium metaperiodate, $RuCl_3.H_2O$, acetonitrile, CCl_4, water, room temperature, 6 hours (c) thionyl chloride, MeOH, (d) Pd/C (10%), H_2, MeOH, 54 hours (e) 2-thiophene ethanol tosylate (7), $NaHCO_3$, dimethyl formamide, reflux, 2 hours (f) methanolic HCl, 1,3-dioxolane, 70 °C, 8 hours (g) (1S)-(+)-camphor-10-sulfonic acid hydrate, acetone, water (h) organic acids, suitable organic acid/solvents.

Among all the organic salts attempted, only the benzene sulfonate salt of compound **(1a)** was obtained in both crystalline and amorphous form while the other salts which could be isolated were obtained in amorphous form. The crystalline benzene sulfonate salt was initially obtained as either the toluene or dioxane solvates; subsequently, it was also possible to prepare the non-solvated form. Since the aim was to prepare the acid addition salts of **(1a)** in crystalline form, the crystalline benzene sulfonate salt was selected for further studies. The stability, dissolution profile of the non-solvated crystalline form was compared with the dioxane and toluene solvates of the benzene sulfonate salt of (**1a**). The efficacy of the non-solvated crystalline form was compared with the dioxane and toluene solvates of the benzene sulfonate salt of (**1a**) in terms of their ability to inhibit the ADP induced (*ex vivo*) platelet aggregation in rat blood sample.

3 Results and discussions

The cyclization of **8** using dioxalane provided the compound **1** in very good yield (~95%). Resolution of **1** using camphor sulfonic acid gave the (+)-(S) isomer (**1a**) with very good chemical and chiral purity. The following organic salts of compound (**1a**) were prepared (Table 1):

Table 1 : Salts of Compound (1a)

Sl. No.	Salt prepared	Melting point	Nature
1.	Mesylate	62-66 °C	Amorphous
2.	p-toluene sulfonate (tosylate)	70-73 °C	Amorphous
3.	sulfosalicylic acid	100-105 °C	Amorphous
4.	Oxalate	92-94 °C	Hygroscopic
5.	Acetate	-	No solid isolated
6.	Gentisate	-	No solid isolated
7.	Napsylate	-	No solid isolated
8.	Benzene sulfonate (from DCM)	85-90 °C	Amorphous
9.	Benzene sulfonate (from THF))	85-90 °C	Amorphous
10.	Benzene sulfonate (from toluene)	86-87 °C	Crystalline (toluene solvate)
11	Benzene sulfonate (from dioxane)	91- 93 °C	Crystalline (dioxane solvate)
12	Benzene sulfonate (from IPA)	130- 135 °C	Crystalline (unsolvated)

The benzene sulfonate salt was only obtained in crystalline form. The pharmaceutical properties, toxicity and efficacy of the non-solvated crystalline benzene sulfonate salt were compared with the dioxane and toluene solvates of the benzene sulfonate salt.

3.1 Comparison of stability of the non-solvated benzene sulfonate of compound (1a) with the toluene-solvate and dioxane-solvate of the benzene sulfonate salt of (1a) under accelerated stability study conditions:

The stability of the three forms of the benzene sulfonate salt (BSA salt) of (**1a**) was studied by loading the APIs in a stability chamber at a temperature of 40 °C ± 2 °C and relative humidity of 75% ± 5%. The samples were analyzed after 3 days, 8 days, 1 month and 2 months and the data is provided in table 2. The non-solvated benzene sulfonate salt remained stable, did not change colour throughout the period of study while, the solvated forms turned to light cream colour. Further, no change in purity was observed for the non-solvated benzene sulfonate salt while there was drop in purity for both the solvated forms.

Table 2: Stability data of solvated and non-solvated forms of BSA salt of (1a) at 40°C ± 2°C temperature and 75% ± 5% relative humidity.

Sr. No	Type of salt	Test	Time period				
			Initial	3 Days	8 Days	1 Month	2 Month
1	Toluene solvate of (1a).BSA	Appearance	Off white colored powder	Off white colored powder	Off white colored powder	Light cream colored powder	Light cream colored powder
		Purity by HPLC	99.85 %	99.77 %	99.62 %	98.30 %	92.63 %
2	Dioxane solvate of (1a).BSA	Appearance	Off white colored powder	Off white colored powder	Light cream colored powder	Light cream colored powder	Light cream colored powder

		Purity by HPLC	99.99 %	99.99 %	99.95 %	99.76 %	99.47 %
3	Non-solvated form of (1a).BSA	Appearance	Off white colored powder	Off white colored powder	Off white colored powder	Off white colored powder	Off white colored powder
		Purity by HPLC	99.88 %	99.88 %	99.93 %	99.67 %	99.82 %

3.2 Dissolution profile and stability data of formulated (1a).BSA non-solvated form, the toluene solvate and the dioxane solvate.

In another study, the non-solvated benzene sulfonate of (**1a**), the toluene and dioxane solvates were each formulated into tablets to study their dissolution profile. The results are depicted in Figure 4. The results clearly demonstrated the superior dissolution profile of the non-solvated form (102.6%) as compared to the solvated forms.

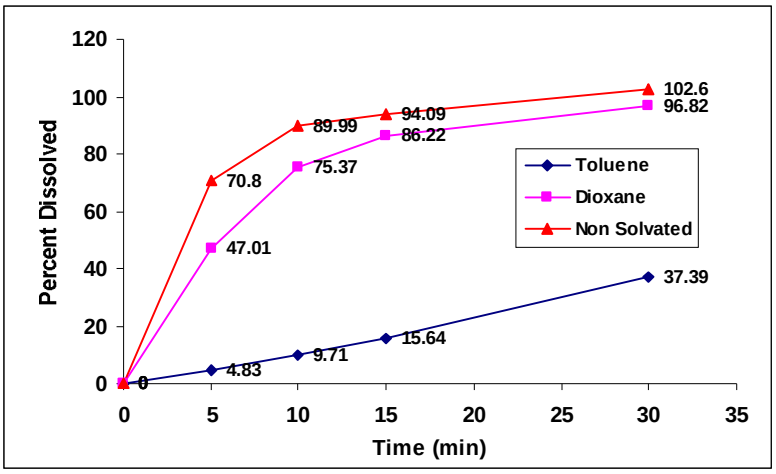


Figure 4: Dissolution profile of the tablets prepared using the three forms of compound of formula (1a) (Initial analysis)

Subsequently these tablets were loaded into a stability chamber at a temperature of 40°C ± 2 °C and relative humidity of 75% ± 5%. Tablets containing the solvated forms of the benzene sulfonate salt of (**1a**) were found to have mottled appearance after 3 days which further increased

towards the end of study. Also dark brown spots developed on the surface of these tablets. The tablets formulated using the non-solvated benzene sulfonate of (**1a**) were found to be absolutely normal in appearance throughout the period of study. Subsequently, purity of the samples was estimated by HPLC and the results are depicted as % fall in purity in Figure 5. The non-solvated benzene sulfonate tablets were purer (97.27%) than the solvated forms at the end of the study.

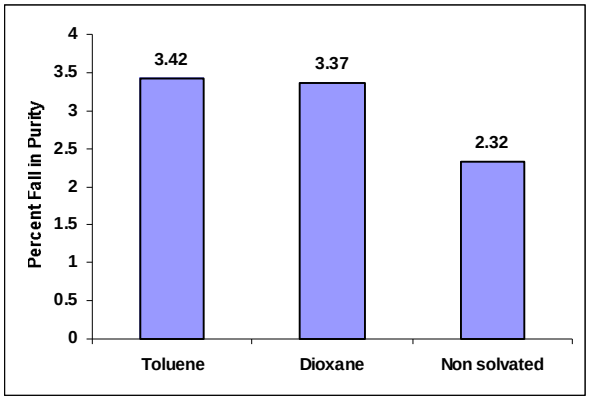


Figure 5: Percent fall in purity during 15 days stability on 40°C/75 RH

The dissolution profile of the three samples was studied again at the end of 15 days of stability test wherein the dissolution profile of the solvated benzene sulfonate tablets were found to have decreased significantly as compared to the non-solvated counterpart on storing for 15 days at a temperature of 40 °C ± 2 °C and relative humidity of 75% ± 5% as shown in Figure 6.

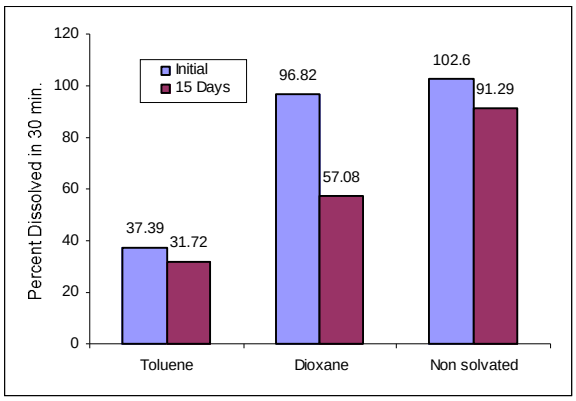


Figure 6: Dissolution results of the non-solvated form, the dioxane-solvate and the toluene solvate forms of the benzene sulfonate salt of compound (1a) tablets kept for stability studies (15 days, 40 °C/75 RH)

In another study, the stability of the non-solvated crystalline benzene sulfonate salt of (**1a**) was studied both under accelerated conditions (40 °C/75% R.H.) and long-term. The salt was found to be stable both under stressed condition and upon long term storage. Further, formation of (+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]-pyridine-5(4H)-acetic acid (the inactive metabolite) was not detected in these studies. Therefore, it is expected that the variability in effectiveness reported for the bisulfate salt (**1b**) will be reduced with this new salt.

The new non-solvated form of the benzene sulfonate salt of (**1a**) was found to have good pharmaceutical properties suitable for further development.

The single crystal of the non-solvated benzene sulfonate salt of formula (**1a**) is given in Figure 7.

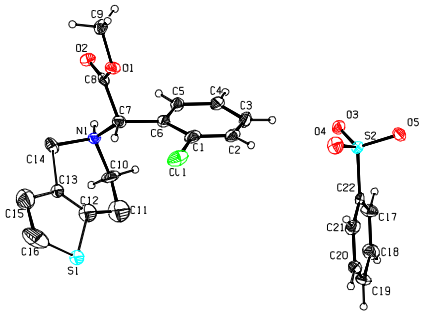


Figure 7: Single crystal analysis of the non-solvated benzene sulfonate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate, showing absence of any solvent molecule.

In a subsequent study the efficacy of the non-solvated benzene sulfonate salt of (**1a**) was compared with bisulfate salt *ex-vivo* and the results are provided in Table 3.

Table 3: Inhibition of ex-vivo platelet aggregation in rats

Conc. Of ADP (µM)	Time (hours)	% Inhibition of platelet aggregation	
		Non-solvated benzene sulfonate salt of (1a)	The bisulfate Form II salt of (1a) (approved drug)
5	2	83 ± 5	64 ± 8
	24	74 ± 8	53 ± 6
	48	60 ± 3	50± 6
10	2	70 ± 6	51 ± 7
	24	62 ± 8	35 ± 9
	48	50 ± 5	35 ± 6
20	2	64 ± 6	44 ± 6
	24	52 ± 8	23 ± 8
	48	40 ± 6	20 ± 5
40	2	72 ± 5	45 ± 7
	24	55 ± 7	34 ± 6

	48	52 ± 5	38 ± 5

From the above table it can be concluded that the non-solvated benzene sulfonate salt of formula (**1a**) showed very good anti-platelet efficacy at all time points and ADP concentrations, with maximum efficacy after 2 hours which reduced gradually after 24 & 48 hours.

4 Conclusion

The novel process using dioxalane for cyclization of **8** gave the final compound (**1a**) in good yields. The process disclosed herein therefore represents an alternate scalable route for the preparation of the compound **1**. Among the various organic salts of compound (**1a**), prepared, only the benzene sulfonate salt was obtained in crystalline form. The benzene sulfonate salt was obtained as solvates of dioxane and toluene as well as a novel non-solvated form. The non-solvated benzene sulfonate salt of compound (**1a**) showed good stability and more importantly, there was no formation of the inactive metabolite, (+)-(S)-(o-chlorophenyl)-6,7-dihydrothieno[3,2-c]-pyridine-5(4H)-acetic acid, in long term storage. Therefore, it is expected that the non-solvated benzene sulfonate salt of compound (**1a**) will be able to overcome the problem associated with the marketed bisulfate.

5 Experimental Section

5.1 Materials and Methods:

Reagents and solvents were obtained from commercial suppliers and used without further purification. Flash chromatography was performed using commercial silica gel (230-400 mesh). Melting points were determined on a capillary melting point apparatus and are uncorrected. IR spectra were recorded on a Shimadzu FT IR 8300 spectrophotometer (V_{max} in cm^{-1}, using KBr pellets or Nujol). The ^1H NMR spectra were recorded on a Brucker Avance-300 spectrometer (300 MHz). The chemical shifts (δ) are reported in

parts per million (ppm) relative to TMS, either in $CDCl_3$ or DMSO-d_6 solution. Signal multiplicities are represented by s (singlet), d (doublet), dd (doublet of doublet), t (triplet), q (quartet), bs (broad singlet), and m (multiplet). ^{13}CNMR spectra were recorded on Brucker Avance-400 at 100 MHz either in $CDCl_3$ or DMSO-d_6 solution. Mass spectra (ESI-MS) were obtained on Shimadzu LC-MS 2010-A spectrometer. HPLC analysis were carried out at λ_{max} 220 nm using column ODS C-18, 150nm * 4.6 nm * 4 μ on AGILENT 1100 series.

5.2 Preparation of Epoxy alcohol (2) from o-chloro cinnamyl alcohol

To 1.5 litres of dichloromethane in a triple neck round bottomed flask was added 50 gm of cinnamyl alcohol at a temperature of 0-5 °C. To the reaction mixture was added 66.54 gms of meta chloro perbenzoic acid with stirring, in lots. The temperature was maintained at room temperature. The reaction mixture was stirred at 0 to 5 °C for 3 hours and the stirring was continued slowly at room temperature overnight. The progress of the reaction was monitored by T.L.C., until all the starting material was consumed. To this reaction mixture was added saturated sodium meta bisulfite aqueous solution, followed by the addition of aqueous NaOH whereby two layers got separated. The organic layer was separated out, while the aqueous layer was re-extracted with 300 ml of dichloromethane. The organic layers was washed with 300 ml. of D. M. water and was dried, concentrated under reduced pressure, and purified by usual procedure yielding the desired 7.0 gm of epoxy alcohol.

5.3 Preparation of 3-azido-3-(o-chloro)-propane-1,2-diol (3) from 2,3-epoxy o-chlorocinnamyl alcohol (2)

20 gm of epoxy alcohol obtained above, was taken in a round bottomed flask and 35.5 gm of NaN_3 and 6.27 gm of NH_4Br was added to it. To the mixture was added 350 ml ethanol and 60 ml water and it was stirred for 2 hours at 75 °C under reflux. The solvent was evaporated when the salt precipitated out of the reaction mixture. To the salt was added 70 ml of dichloromethane, filtered and the residue was rejected. To the filtrate was added 100 ml of

water when two layer got separated. The lower MDC was separated, and dried. The solvent was evaporated at 40 °C under reduced pressure to give 20.2 gm of the diol.

IR (cm^{-1}) ($CHCl_3$): 3384 (-OH stretch); 2106 (N_3)

^{1}HNMR ($CDCl_3$): δ 1.96 (t, 1H), 2.4 (d, 1H); 3.7 (t, 2H), 3.9-4.0 (q, 1H); 5.2 (d, 1H), 7.2-7.5 (m, 4H).

^{13}CNMR ($CDCl_3$): δ ppm 62.56, 63.73, 73.06, 127.4, 128.7, 129.7,130.02, 133.7, 133.91.

MS : m/z 288.1 $(M+H)^+$, 245.2 $(M+NH_4)^+$

5.4 Preparation of azido acid (4) from 3-Azido-3-(o-chloro phenyl) propane-1,2-diol (3).

In a 1 litre round bottomed flask was taken 11 gm of the azidodiol obtained above and to it added 92 ml of acetonitrile, 87 ml of CCl_4 and 131 ml. D. M. water, when two layer was formed. To the mixture was added 4.0 eq. sodium metaperiodate and 236 mg of $RuCl_3.H_2O$, when the colour changed to brick red. The reaction mixture was stirred for 6 hours at room temperature and kept overnight. To the mixture was added 200 ml of ether, the organic layer was filtered out through hyflow bed, washed with water and dried over anhydrous Na_2SO_4 to give 6.0 gm (64.5%) of azido acid.

IR (cm^{-1}) ($CHC1_3$): 2928 (NH), 2110 (N_3), 1725 (CO)

^{1}H NMR ($CDC1_3$): 5.59 (s, 1H), 7.3-7.36 (m, 2H), 7.42-7.48 (m, 2H), 9.5 (b, 1H)

^{13}C NMR ($CDC1_3$): δ ppm 61.99, 127.5, 128.87, 130.19, 130.7, 131.5, 134.02, 173.8.

MS: m/z 210 $(M+H)^+$

5.5 Preparation of azido ester (5) from corresponding azido acid (4):

1.0 gm of azido acid obtained above was taken in a round bottomed flask and 5 ml of methanol was added to it and the solution was stirred for 30 minutes. To the reaction mixture was added 0.9 grams of thionyl chloride drop wise. The reaction mixture was stirred for 3 hours at room

temperature. The excess solvent was evaporated under reduced pressure. To the residue was added 25 ml MDC, the organic layer formed was washed with a 1% solution of $NaHCO_3$ and then with D. M. water and the organic layer was dried with anhydrous Na_2SO_4. The excess solvent was evaporated under reduced pressure when the azido ester was obtained in ~ 90% yield (0.9 gm).

IR (cm^{-1}): 2108 (N_3), 1751.2 (CO)

^1H NMR ($CDC1_3$): 3.79 (s, 3H), 5.5 (s, 1H), 7.31-7.46 (m, 4H);

^{13}C NMR ($CDC1_3$): δ ppm 53.03, 62.02, 62.02, 127.3, 128.7, 130.0, 130.4, 132.05, 133.09, 169.06.

MS : m/z 243.2 $(M+NH_4)^+$

5.6 Preparation of amino ester (6) by reduction of azido ester (5) using Palladium on charcoal

1gm of azido ester prepared above was dissolved in 20 ml MeOH and added to the metallic container of Parr apparatus. To it was added Palladium charcoal (10%) and the container was shaken for 54 hours under hydrogen pressure. After completion of the reaction, the reaction mixture was filtered, the solvent evaporated under reduced pressure and 25 ml of MDC added to the reaction mixture. The amino ester obtained was purified by conventional techniques, when 250 gm (28.4%) of the purified product was obtained.

IR (cm^{-1}) : 3381 (NH-stretch), 1738 (CO), 1126 (CN stretch)

^1H NMR ($CDC1_3$): δ 2.0 (bs 2H), 3.72 δ (s, 3H, OCH_3), 5.0δ (s, 1H), 7.24-7.28 (m, 2H), 7.33-7.40 (m, 2H)

MS : m/z 200 $(M+H)^+$

5.7 Preparation of the compound of formula (8) by coupling 2-thiophene ethanol tosylate (7) with amino ester derivative (6)

4gm of the amino ester (6) obtained previously was taken in a round bottomed flask & to it was added 1.4 eq. of 2-thiophene ethanol tosylate (7), 20 ml of dimethyl formamide and 2 eq. of $NaHCO_3$. The mixture was refluxed for 2 hours, kept overnight and the excess solvent was evaporated

under reduced pressure. The crude product was purified using conventional techniques to obtain 0.9gm of (**8**).

IR (cm^{-1}): 3018.4 (NH stretch), 1736 (CO)

^1H NMR ($CDC1_3$): δ 2.1 (b, 1H), 3.0 (m, 1H), 3.3 (m, 1H), δ 3.5-3.6 (m, 2H), 5.65 (s, 1H), 6.8 (d, 1H), 6.9 (dd, 1H), 7.1 (dd, 1H), 7.3-7.4δ (m, 3H), 8.0 (dd, 1H);

^{13} C NMR: δ ppm 30.44, 48.89, 52.33; 61.5, 123.5, 124.9, 126.7, 127.2, 128.5, 129.1, 129.7, 134.0, 136.0 and 142, 172.59.

MS : m/z 310.2 $(M+H)^+$

5.8 Cyclization of open chain compound (8) to (1)

To 2.0 gm of the HCl salt of the ester (8) obtained above was added 5 ml of 1,3-dioxolane and 0.5 ml of methanolic HCl. The mixture was stirred for 8 hours at 70 °C, the excess dioxolane was evaporated under reduced pressure. The residue was dissolved in 60 ml dichloromethane, basified with 10% $NaHCO_3$, the organic layer was washed with water and the excess solvent was evaporated under reduced pressure to obtain 1.68 gm (~ 95%) of compound of formula (**1**) as free base.

IR (Cm^{-1}): 1740 (CO);

^1H NMR ($CDC1_3$): δ 2.88 (s, 4H), 3.6-3.8 (s, 3H, m, 2H), 4.9 (s, 1H), 6.6 (d, 1H), 7.0 (d, 1H), 7.2-7.7 (m, 4H);

^{13} C NMR ($CDC1_3$): 25.54, 48.3, 50.6, 52.14, 67.8, 122.7, 125.2, 127.1, 129.4, 129.7, 129.9, 133.2 133.8, 134.7 and 171.34;

MS : m/z 322.1 $(M+H)^+$

5.9 Preparation of (S)-(+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

2 g (0.0173 mol.) of Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate was dissolved in 10 ml acetone and the reaction mixture was stirred for 10 min, followed by reflux. To the reaction mixture, 1.49 g (1S)-(+)-camphor-10-sulfonic acid hydrate in 0.8 mL water was added followed by 1 mL acetone. Then whole reaction mixture was refluxed for 1

hr. and cooled gradually. This was later stirred overnight at room temperature. The clear solution was cooled further at 0 to -5 °C, wherein precipitate was obtained. The salt formed was added to ethyl acetate and water, which was later basified with $NaHCO_3$, the organic layer was washed with water, concentrated under reduced pressure, to give free base 0.386 g with chiral purity = 99.85 % (+)-isomer (e.e. = 99.7 %).

IR (cm^{-1}): 1740 (CO)

^1H NMR ($CDC1_3$): δ 2.88 (s, 4H), 3.6-3.8 (s, 3H, m, 2H), 4.9 (s, 1H), 6.6 (d, 1H), 7.0 (d, 1H), 7.2-7.7 (m, 4H);

^{13}C NMR ($CDC1_3$): 25.54, 48.3, 50.6, 52.14, 67.8, 122.7, 125.2, 127.1, 129.4, 129.7, 129.9, 133.2 133.8, 134.7 and 171.34

MS: m/z 322.1 $(M+H)^+$

5.10 Preparation of mesylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

The compound of formula (1a) was dissolved in methanol. To it 20 gm of methane sulfonic acid was added. The reaction mixture was refluxed for 30 hours and the methanol was distilled off under vacuum to provide the mesylate salt in amorphous form.

m. p: 60-70 °C (soften)

XRD: Amorphous

DSC: No melting peak

IR (KBr, cm^{-1}): 1743, 1649, 1510, 1434, 1155 & 1042

5.11 Preparation of mesylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

19.14 gm of compound (1a) was dissolved in 200 ml. of acetone. To the solution was added 3.86 ml. of methane sulfonic acid in one lot. The mixture was refluxed with stirring for 2 hours and then distilled off under reduced pressure at 50-55 °C. It was dried at 40-50 °C for 45 minutes to obtain the mesylate salt.

Weight: 23 gms

M.P. = 60-64 °C.

XRD: Amorphous

% water: 2.4%.

5.12 Preparation of mesylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

To 3.8 gm of compound (1a) was added 40 ml of acetone and stirred to dissolve. To the solution was added 0.76 ml of methane sulfonic acid in one lot. The mixture was stirred and refluxed for 2 hours and then cooled to room temperature. No solid was formed. Subsequently, the solvent was distilled off under reduced pressure at 50-55 °C, scratched and dried to obtain the mesylate salt.

Weight: 4.7 gms

M.P. = 62-66 °C

XRD: amorphous

% water: 3.60%

IR: 1751.2 cm^{-1}

5.13 Preparation of mesylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

To 3.8 gm of compound (1a) was added 45 ml of acetone and stirred to dissolve. To the solution was added methane sulfonic acid 0.76 ml (1 mole equivalent) in one lot. The mixture was stirred and refluxed for 1 hour and then cooled to 25-30 °C. No solid was obtained. The solvent was distilled off under reduced pressure to obtain a sticky mass. It was dissolved in 30 ml of DCM and then the solvent was distilled off. Then high pressure was applied when a solid mass was obtained upon scratching. However, the solid became hygroscopic on standing. It was dissolved in DIPE and kept for crystallization. No solid was obtained.

The reaction was carried out using other solvents such as toluene, THF, acetonitrile wherein the mesylate salt was always obtained in amorphous form. None of the reactions yielded crystalline mesylate salt of (1a).

5.14 Preparation of p-toluene sulfonate (tosylate) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.2 gm of p-toluene sulfonic acid and 5.5 gm of the compound of formula (1a) was dissolved in acetonitrile at RT. It was refluxed for 20 hours and then the solvent was removed by heating when 8.7 gm of the toluene sulfonate salt.

m.p. 75 °C;

DSC endothermic-no melting peak;

XRD: amorphous.

5.15 Preparation of p-toluene sulfonate (tosylate, p-TSA) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gm of compound (1a) was dissolved in 20 ml of diethyl ether and 9.24 gm of p-TSA was dissolved in 40 ml of diethyl ether with stirring and to it was added the solution of compound (1a). A sticky material was obtained. Part of it was added to pet ether, and stirred, no solid was obtained. The rest of the sticky mass was dissolved in acetone (40 ml) and the solvent was distilled off in vacuum. The solid obtained was dried at 25-30 °C. The solid became sticky after some time and was difficult to handle.

5.16 Preparation of p-toluene sulfonate (tosylate) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

7.6 gm of compound (1a) was dissolved in 70 ml of acetone. To it was added 9.5 gm of p-toluene sulfonic acid and the mixture was refluxed for 1 hour and the solvent was distilled off. The residue was stirred in 60 ml of diisopropyl ether (DIPE) for 15 minutes, filtered and washed with DIPE to obtain a solid. Part of the solid became sticky and so the solid was dissolved in acetone 7.0 ml and refluxed for 15 minutes and then the solvent was distilled off to get a solid upon applying high vacuum for 30-40 minutes.

Weight of solid: 9 gms

M.P. = 70-73 °C.

XRD: amorphous

5.17 Preparation of p-toluene sulfonate (tosylate) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gms of compound (1a) was dissolved in 40 ml of diisopropyl ether (DIPE). To it was added 2.24 gm of Tosic acid.H_20 and the mixture was stirred in a water bath at 70-72 °C for 1 hour when an oily layer separated. The solution was stirred with intermittent scratching for 1 hour when a sticky solid was obtained. This solid was dissolved in 50 ml of acetone when a clear solution was obtained. The solvent was distilled off and high vacuum was applied for 1 hour at 25-30 °C. A solid mass was obtained which again became sticky when kept overnight. The product was again dissolved in 60 ml of DIPE, stirred for 30 minutes, filtered, washed with DIPE, suck dried and then dried at 50-60 °C for 1 hour to give an off-white powder.

Weight of solid: 5.67 gms

M.P. = 75-76 °C

XRD: amorphous

DSC: no peaks.

5.18 Preparation of p-toluene sulfonate (tosylate) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

a) 3.8 gms of compound (1a) was dissolved in 25 ml of acetone and to it was added 2.24 gm (1 mole eq.) of Tosic acid.H_20 when a clear solution was obtained. It was stirred for 23 hours at room temperature. The solvent was distilled off on a water bath at 50-52 °C under reduced pressure, scratched and then vacuum was applied when a solid was obtained.

   Weight of solid: 6 gms

   M.P. = 67-72 °C

   XRD: amorphous

IR: 1751 cm^{-1}

b) 1 gm of the product obtained in step (a) was dissolved in 10 ml of diisopropyl ether and stirred for 15 minutes at 25-30 °C. The mixture was filtered and washed with minimum amount of DIPE and then dried at 40-42 °C.
Weight of solid: 900 mg
XRD: amorphous

c) 500 mg of the product obtained in step (a) was dissolved in 5 ml of acetone and then precipitated by addition of 10 ml of DIPE. The mixture was stirred, scratched, filtered and dried.
Weight of solid: 300 mg
XRD: amorphous

Therefore, attempts to prepare the crystalline p-TSA salt of compound (1a) were not successful and the salt was always obtained in amorphous form.

5.19 Preparation of sulfosalicylic acid (SSA) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gms of compound (1a) was dissolved in 25 ml of MeOH and to it was added 3 gms of 5-sulphosalicylic acid and the mixture was stirred for 40 minutes and then cooled. No solid separated. The mixture was refluxed for 1 hour and then cooled to 25-30 °C and the solvent was distilled off to get a solid.

Weight of solid: 6.1 gms

% water: 3.71 %

M.P. 70-75 °C

XRD: Amorphous

5.20 Preparation of sulfosalicylic acid (SSA) salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

a) 3.8 gm of compound (1a) was dissolved in 25 ml of acetone and to it was added 3 gm of 5-sulfosalicylic acid in one lot. The clear solution obtained was stirred at 25-30 °C for 30 minutes, no solid separated

out. The solution was then refluxed for 45 minutes, cooled and stirred at 25-30 °C for another 30 minutes. The solvent was distilled off, and the solid was taken in 25 ml of DCM, stirred and distilled under normal pressure. Traces of the solvent were removed under reduced pressure.

Weight of the solid: 6.4 gms

% water: 2.16%

M.P. = 100-105 °C (decomposed)

XRD: Amorphous

b) 1 gm of the compound obtained in step (a) above was stirred in ethyl acetate for 60-65 minutes. The solution was filtered, washed with minimum (2 ml) of ethyl acetate, dried at 50-52 °C.

Weight of solid: 759 gms

M.P. = 102-105 °C

XRD: Amorphous

5.21 Preparation of oxalate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gms of compound (1a) was dissolved in 40 ml of acetone and to it was added 1.57 gm of aspartic acid which remained un-dissolved. The mixture was heated at 60-62 °C on a water bath. The solvent was then distilled off, cooled, added water, scratched and then added 50 ml of acetone. Still no clear solution was obtained and no solid could be isolated.

5.22 Preparation of oxalate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

20 gm of compound of formula (1a) and 6.3 gm of oxalic acid are dissolved in 130 ml of dichloromethane. The solution was refluxed for 40 hours. A sticky solid was obtained which could not be used for characterization.

5.23 Preparation of oxalate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gm of compound (1a) was dissolved in 25 ml of acetone and to it was added 1.49 gm of oxalic acid.$2H_2O$ in one lot when a clear solution was obtained. The solution was stirred-refluxed for 6 hours and the solid was distilled off to get a low melting solid which was difficult to isolate and characterize. The product was dissolved in 100 ml of DCM. The solvent was distilled off under atmospheric pressure. Again a pasty material was obtained. The mass was dissolved in 30 ml of pet ether, filtered, washed to get a sticky solid. It was taken in 30 ml acetone, dissolved, the solvent distilled off, scratched and the mass again taken in pet ether and stirred for 1 hour. It was filtered and washed with pet ether and dried.

Weight of solid: 2.8 gm

M.P. 92-94 °C (hygroscopic)

5.24 Preparation of oxalate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gms of compound (1a) was dissolved in 24 ml of acetone and to it was added 2.49 gm of oxalic acid.$2H_2O$ (1 mol equivalent) in one lot. The clear solution was stirred at 25-30 °C for 24 hours. The solvent was distilled off and a sticky solid was obtained. To it was added 24 ml of DCM, stirred and solvent distilled off at atmospheric pressure. A sticky material was obtained. This was stirred in 90 ml of pet ether at 60-80 °C, when again a sticky solid separated. This was again dissolved in DCM and then distilled; the solid was stirred again in 20 ml of pet ether, filtered and dried.

Weight of solid: 2.5 gm

M.P. = 90-92 °C (hygroscopic).

The following salts were also attempted: acetate, napsylate, gentisate. However, they could not be isolated in solid form.

5.25 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

19.14 gms of compound (1a) was dissolved in 151 ml of acetone and a clear solution was obtained. To it was added a solution of benzene sulfonic acid (9.4 gms) in acetone (40 ml). The mixture was stirred with reflux for 2 hours. Then the solvent was removed under reduced pressure on a water bath at 50-55 °C. To it was added 100 ml of DCM and the solvent was distilled off under atmospheric pressure. Subsequently high vacuum was applied first at 50-55 °C and then at 20-25 °C. The solid was scratched and dried at 65-70 °C for 30 minutes.

Weight of solid: 27.2 gms

M.P. = 85-90 °C

XRD: amorphous

5.26 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

The compound of formula (1a) was dissolved in THF. To it, benzene sulfonic acid was added at 20 °C, and the reaction mixture was heated to reflux temperature for 10 hrs. The solvent was evaporated to dryness under reduced pressure to obtain the besylate, salt of formula (1a) in the amorphous form.

M.P.: 92 ± 3 °C;

XRD: Amorphous;

DSC: No melting peak

% water: 2.5 % by weight

5.27 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.8 gms of compound (1a) was dissolved in 30 ml of acetone and to it was added 1.86 gm of benzene sulfonic acid in 8 ml of acetone. The mixture was refluxed for 2 hours and then the solvent was distilled off under reduced

pressure at 50-55 °C on a water bath. High vacuum was applied subsequently at 25-30 °C for 30-40 minutes. The solid was scratched and dried at 50-55 °C for 1 hour.

Weight of solid: 5.2 gms

M.P. = 85-95 °C

% water: 1.85%

IR: 1751.2 cm^{-1}

XRD: amorphous

5.28 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate from acetone/toluene (1a)

i) 4.0 gm (12.5 mmol) of compound (1a) was dissolved in toluene (30 ml) and 2 gm (12.5 mmol) of anhydrous benzene sulfonic acid in acetone (10 ml) was added to it. It was kept for some time and scratched with a glass rod, when the product was obtained as solid which was filtered through suction. The solid was dried overnight in a desiccator attached to a vacuum pump.

Weight of solid: 3.92 gms

M.P. = 86-87 °C; by DSC: onset: 81 °C, peak: 90 °C.

^{1}H NMR: 2.42 (toluene), 3.07-3.55 and 4.02-4.40 (4H), 3.85 (3H), 4.87-5.21 (1H), 5.76 (1H), 6.70-6.86 (1H), 7.25-8.04 (10H)

XRD: Crystalline

The experiment was repeated once. The data are provided below:

ii) Weight of solid: 3.95 gms
M.P. = 87-88 °C
^{1}H NMR: 2.42 (toluene), 3.07-3.55 and 4.02-4.40 (4H), 3.85 (3H), 4.87-5.21 (1H), 5.76 (1H), 6.70-6.86 (1H), 7.25-8.04 (10H)
XRD: Crystalline

The product obtained was the toluene solvate.

5.29 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate from dioxane(1a)

i) A solution of 5.37 gm (33.97 mmol) of anhydrous benzene sulfonic acid in dioxane (10 ml) was added to 10.92 g (33.97 mmol) of compound (1a) in dioxane (35 ml) with stirring at 10 °C. Ethyl acetate (20 ml) was added to the solution and the mixture was kept in a deep fridge overnight. The solution was allowed to come to room temperature; adsorbates were removed by filtration and washed with ethyl acetate. The product was dried in vacuo at room temperature for 48 h.

Weight of solid: 13.11 gms

M.P. = 91- 93 °C; by DSC: onset: 85.4 °C, peak: 96.4 °C

^{1}H NMR: 2.97-3.44 and 3.82-4.26 (4H), 3.78 (3H), 4.77-5.11 (1H), 5.67 (1H), 6.61-6.77 (1H), 7.23-7.93 (10H), 3.67 (4H, ½ dioxane)

XRD: Crystalline

The experiment was repeated once. The data is provided below:

ii) Weight of solid: 13.11 gms

M.P. = 90- 91 °C.

^{1}H NMR: 2.97-3.44 and 3.82-4.26 (4H), 3.78 (3H), 4.77-5.11 (1H), 5.67 (1H), 6.61-6.77 (1H), 7.23-7.93 (10H), 3.67 (4H, ½ dioxane)

XRD: Crystalline

The product obtained was the dioxane solvate.

5.30 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

The base of formula (1a) (60 g) was dissolved in isopropanol at 50-55 °C. To it was added benzene sulfonic acid (30 g) dissolved in isopropanol at 50-55 °C. The reaction mixture was stirred for 20 hr. The solid was filtered and washed with isopropanol and dried in a vacuum oven for at least 20 hr. to

give the besylate salt of formula (I), which on characterization was found to be crystalline form.

M.P. 130-135 °C;

m.p.: 126-130 °C;

XRD: Crystalline;

DSC: 130.5 – 135.9 °C.

5.31 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

The base of formula (1a) (5 g) was dissolved in methyl tertiary butyl ether. To this, benzene sulfonic acid (2.5 g) dissolved in methyl tertiary butyl ether was added at 50-55 °C. The reaction mixture was seeded with crystalline besylate salt of formula (I) (50 mg) and the reaction mixture was stirred for at least 24 hr. The solid was filtered and washed with methyl tertiary butyl ether and dried in vacuum oven for at least 20 hr. to give besylate salt of the compound of formula (1a), which on characterization was found to be crystalline form.

M.P. 135.1± 2 °C.

XRD: Crystalline

5.32 Preparation of besylate salt of (S)-(+) (+)-Methyl (2-chlorophenyl)-(6,7-dihydro-4H-thieno[3,2-c]pyrid-5-yl)acetate (1a)

3.82 gms of compound (1a) was dissolved in 25 ml of diethyl ether with continuous stirring. Separately 2gm of benzene sulfonic acid was dissolved in 25 ml of diethyl ether and this solution was added drop-wise to the solution of the base (1a) over 10 minutes at 25-30 °C. The solution was stirred for 22-24 hours when solid separated. It was filtered, and the solid was dried at 60-65 °C.

Weight of solid: 1.79 gms

XRD: Crystalline

M.P. = 135-138°C

DSC: 129 (onset) – 132.7 (peak)

DSC, TGA and single crystal analysis conclusively proved that the benzene sulfonate salt of the compound of formula (1a) prepared according to the present process is a non-solvated form.

5.33 Biological studies

5.33.1 Evaluation of efficacy of the crystalline form of the besylate salt of formula (1a) with the marketed bisulfate salt (Form 2) at various time points in inhibiting ADP induced (*ex vivo*) platelet aggregation in the rats.

Materials and methods:

i. Test item details:

Sl. No.	Salt name	Purity (%)	Melting point (°C)
1.	Crystalline besylate of formula (1a)	99.5	136.6
2.	Clopidogrel bisulfate salt (Form II)	99.6	176

ii. Dose formulation

The salt forms were formulated in saline containing 5% ethanol and each rat received 5 ml/kg p.o. volume.

iii. Storage conditions

Solutions of test compounds were always prepared fresh before use and were not stored.

iv. Test system & Experimental Design

Species: Wister Rat

Sex: Male

Body weight range: 200-250 g

Age of initiation: 6-8 weeks

Route: Oral

Frequency of dosing: Once

Volume of administration: 5 ml/Kg

Duration of study: 10 days

Blood collection: 2 hours, 24 hours and 48 hours post treatment (different set of animals were used for each time point)

v. Group and dosage levels

Group	Treatment	Dose	No. of animals
I	Vehicle control	-	6+6+6
II	Crystalline besylate of compound of formula (1a)	10*	6+6+6
III	Clopidogrel bisulfate (Form 2)	10*	6+6+6

* Equivalent to the base

vi. Methodology (Ref: *Journal of Thrombosis and Haemostasis, 2007, 5, 1545-1551).*

The formulations of the salts were always prepared freshly in vehicle. The vehicle (10 mg/kg/5 ml) were administered to non-fasted rats. At 2, 24 & 48 h after initial dosing, 8 ml of blood was collected by cardiac puncture (after anaesthetizing the rats) from which the platelet rich plasma was isolated and the platelet aggregation was measured using 2.5 % (w/v) sodium citrate was used as the anti-coagulant.

The main pharmacodynamics measure was the inhibition/reduction of ADP-induced platelet aggregation of platelet-rich plasma (PRP) quantitated using optical density filter at 405 nm as a measurement point in kinetic mode. To obtain PRP, a citrated tube of blood was inverted 3 to 5 times for gentle mixing and centrifuged at room temperature for 10 minutes at 200g. After centrifugation, the upper turbid layer of PRP was removed, and the residual blood was centrifuged for 5 minutes at 2000 g to obtain platelet-poor plasma (PPP). The PPP was used as the baseline optical density for platelet aggregation. A total of 180 µL of PRP containing 3 x 10^8 platelet/ml was incubated at 37 °C in the 96 well plate for 3-5 minutes, followed by the addition of ADP (20 µL) at a final concentration of 10, 20 & 40 µmol/L with

intermittent shaking mode. Optical density readings were measured every 1-minute with intermittent shaking up to 5 minutes. Platelet aggregation was expressed as the change in optical density at 5 minutes, compared with PPP as a reference and converted to % aggregation.

vii. Calculations and statistical evaluation

Statistical analysis of % aggregation was performed by one-way ANOVA (Analysis of Variance) followed by Bonferroni post hoc test. All these analyses were done using Graph pad Prism software. Results are expressed as mean ± SEM and differences of $P<0.05$ were considered statistically significant.

6 References:

1. Yusuf S, Zhao F, Mehta S.R., Chrolavicius S, Tognoni G, Fox K.K., Clopidogrel in Unstable Angina to Prevent Recurrent Events Trial Investigators., Effects of Clopidogrel in Addition to Aspirin in Patients with Acute Coronary Syndromes without ST-Segment Elevation *N Engl J Med,* **2001**,345: 494–502.

2. CAPRIE Steering Committee, A randomised, blinded, trial of clopidogrel versus aspirin in patients at risk of ischaemic events (CAPRIE). CAPRIE Steering Committee, *Lancet*, **1996**, 348:1329–1339.

3. Savi P., Labouret C., Delesque N., Guette F., Lupker J., Herbert J.M.,P2y(12), a new platelet ADP receptor, target of clopidogrel, *Biochem Biophys Res Commun*, **2001**,283:379–383.

4. Creager M.A., Results of the CAPRIE trial: efficacy and safety of clopidogrel. Clopidogrel versus aspirin in patients at risk of ischaemic events, *Vasc Med.,* **1998**, 3:257–260.

5. Savi P, Herbert J.M., Pflieger A.M., Dol F, Delebassee D, Combalbert J, Defreyn G, Maffrand J.P., Importance of hepatic metabolism in the antiaggregating activity of the thienopyridine clopidogrel, *Biochem Pharmacol.*, **1992**, 44:527–532.

6. Savi P., Pereillo J.M., Uzabiaga M.F., Combalbert J., Picard C., Maffrand J.P., Pascal M., Herbert J.M., Identification and biological activity of the active metabolite of clopidogrel, *Thromb Haemost,* **2000**, 84: 891-896.

7. Savi P., Combalbert J., Gaich C., Rouchon M.C., Maffrand J.P., Berger Y., Herbert J.M., The anti-aggregating activity of clopidogrel is due to a metabolic activation by the hepatic cytochrome P450-1A, *Thromb Haemost,* **1994,** 72: 313–317.

8. Clarke T.A., Waskell L.A., The metabolism of clopidogrel is catalyzed by human cytochrome P450 3A and is inhibited by atorvastatin, *Drug Metab Dispos,* **2003**, 31: 53–59.

9. Hulot J.S., Bura A., Villard E., Azizi M., Remones V., Goyenvalle C., Aiach M., Lechat P., Gaussem P., Cytochrome P450 2C19 loss-of-function polymorphism is a major determinant of clopidogrel responsiveness in healthy subjects, *Blood,* **2006**, 108:2244–2247.

10. Suh J.W., Koo B.K., Zhang S.Y., Park K.W., Cho J.Y., Jang I.J., Lee D.S., Sohn D.W., Lee M.M., Kim H.S., Increased risk of atherothrombotic events associated with cytochrome P450 3A5 polymorphism in patients taking clopidogrel, *CMAJ,* **2006**, 174:1715–1722.

11. Brandt J.T., Close S.L., Iturria S.J., Payne C.D., Farid N.A., Ernest C.S., Lachno D.R., Salazar D., Winters K. J., Common polymorphisms of CYP2C19 and CYP2C9 affect the pharmacokinetic and pharmacodynamic response to clopidogrel but not prasugrel, *J Thromb Haemost,* **2007**,5: 2429-2436.

12. Farid N.A., Payne C.D., Small D.S., Winters K.J., Ernest C.S., Brandt J.T., Darstein C., Jakubowski J.A., Salazar D.E., Cytochrome P450 3A inhibition by ketoconazole affects prasugrel and clopidogrel pharmacokinetics and pharmacodynamics differently, *Clin. Pharmacol. Ther.,* **2007**, 81:735–741.

13. Farid N.A., Small D.S., Payne C.D., Jakubowski J.A., Brandt J.T., Li Y. G., Ernest C.S., Salazar D.E., Konkoy C.S., Winters K.J., Effect of

atorvastatin on the pharmacokinetics and pharmacodynamics of prasugrel and clopidogrel in healthy subjects, *Pharmacotherapy,* **2008**, 28: 1483–1494.

14. Sung-Doo K, Wonku, K., Lee H.W., Park D.J., Kim M.J., Bioequivalence and tolerability of two Clopidogrel salt preparations, besylate and bisulfate: A randomized, open-label, crossover study in healthy Korean male subjects, *Clin. Therap.*, **2009,** 31: 793-803.

15. Sadhukhan A., Saravanan S., Khan Noor-ul H., Kureshy R.I., Abdi S.H.R., Bajaj H.C., Modified Asymmetric Strecker reaction of aldehyde with secondary amine: A protocol for the synthesis of S-Clopidogrel (an antiplatelet agent), *J Org. Chem.*, **2012**, 77: 7076-80.

16. Aillaud I., Haurena C., Gall E.L., Martens T., Ricci G.,2-Chlorophenyl Zinc Bromide: A convenient nucleophile for the Mannich-related multicomponent synthesis of Clopidogrel and Ticlopidine, *Molecules (Basel, Switzerland)*, **2010**,15: 8144-55.

17. Ema T, Okita N, Ide S., Sakai T., Highly enantioselective and efficient synthesis of methyl (R)-o-chloromandelate with recombinant E. coli: toward practical and green access to clopidogrel, *Org. & Biomol. Chem.*, **2007,** 5: 1175-6.

18. Jeong M., Lee Y.M., Hong S.H., Park S.Y., Yoo I.K., Han M.J., Optimization of enantioselective synthesis of methyl (R)-2-chloromandelate by whole cells of *Saccharomyces cerevisiae*, *Biotech. Lett.*, **2010**, 32: 1529-31.

19. Chenghai Y., Preparation process of clopidogrel and its salt, CN 100999525

20. Vaghela M.N., Rehani R.B., Thennati R., A process for preparation of clopidogrel, WO 2004108665.

21. Simon L., Chenq-Chunq C., Process for preparation of 2-chlorophenylglycine derivatives and enantiomerically separation, US 20040176637.

22. Bakonyl M., Csatarine N.M., Molnar L., Gajary A., Alattiani E., A new process for the preparation of a pharmacologically active substance, WO 9851689.

23. Lu Y., Wenjun S., Xian J., Xingshu L., Albert S.C.C., Ru-catalyzed enantioselective preparation of methyl (R)-o-chloromandelate and its application in the synthesis of (S)-Clopidogrel, *J. Organometallic Chem.,* **2009**, 694: 2092-2095.

24. Aubert D., Ferrand C., Maffrand J., Thieno(3,2-c) pyridine derivatives, process for their preparation and their therapeutical use, EP 99802.

25. Bouisset M., Radisson J., Process for preparing phenyl acetic derivatives of thienopyridines and intermediates alpha-bromo-phenyl acetic acids, EP420706.

26. Jiaqi S., Boyu Z., Yaoqiu Z., Bo J., Zheng W., Xiaowei Q., Yanchun G., Fang Y., Fusheng L., Hongbin S., Overcoming Clopidogrel resistance: discovery of Vicagrel as a highly potent and orally bioavailable antiplatelet agent, *J Med. Chem.*, **2012**, 55: 3342-52.

27. Achilleas M., Irene P., Determination of the carboxylic acid metabolite of clopidogrel in human plasma by liquid chromatography-electrospray ionization mass spectrometry, *Analytica Chimica Acta,* **2004**, 505: 107-114.

28. Hanna K., Piotr R., Bukowska-Kiliszek M., Determination of clopidogrel metabolite (SR26334) in human plasma by LC–MS, *J. Pharm. Biomed. Anal.,* **2006**, 41: 533-539.

29. Gomez Y., Adams E., Hoogmartens J, Analysis of purity in 19 drug product tablets containing clopidogrel: 18 copies versus the original brand, *J Pharm. Biomed. Anal.*, **2004**, 34: 341-8.

30. Khan S.B., Hameedullah N.L., Hafeezullah M., Awan Z.A., Din S.U., Comparison of effect of locally available brands of Clopidogrel on platelet aggregation in patients with coronary artery disease, *Journal of Ayub Medical College, Abbottabad : JAMC*, **2010**, 22:115-7.

31. Badorc A., Frehel D., Dextro-rotatory enantiomer of methyl alpha-5 (4,5,6,7-tetrahydro (3,2-c) thienopyridyl) (2-chlorophenyl)-acetate and the pharmaceutical compositions containing it, US 4847265.

32. Doser K.H., Glänzer K., Salz der benzolsulfonsäuremit clopidogrel und dessenverwendungzurherstellung pharmazeutischer formulierungen, EP 1480985.

33. Park J.B., Koo B.K., Choi W.G., Kim S.Y., Park J., Kwan J., Park C.G., Kim H.S., Comparison of antiplatelet efficacy and tolerability of Clopidogrel napadisilate with Clopidogrel bisulfate in coronary artery disease patients after percutaneous coronary intervention: A prospective, multicenter, randomized, open-label, phase IV, non-inferiority trial, *Clin. Therap.*, **2013**, 35: 28-37.

34. Sambu N., Radhakrishnan A., Curzen N., A Randomized crossover study comparing the antiplatelet effect of Plavix versus generic Clopidogrel, *J Cardiovasc. Pharmacol.*, **2012**, 60:495-501.

35. Kim Y.I., Kim K.S., Suh K.H., Shanmugam S., Woo J.S., Yong C.S., Choi H.G., New clopidogrel napadisilate salt and its solid dispersion with improved stability and bioequivalence to the commercial clopidogrel bisulphate salt in beagle dogs, *Int. J Pharm.*, **2011**, 415:129-39.

36. Ki M.H., Choi M.H., Ahn K.B., Kim B.S., Im D.S., Ahn S.K., Shin H.J., The efficacy and safety of clopidogrel resinate as a novel polymeric salt form of clopidogrel, *Arch. of Pharmacol. Res.*, **2008**, 31: 250-8.

37. Caron M., Carlier P.L., Sharpless K.B., Regioselective azide opening of 2,3-epoxy alcohols by [Ti(O-i-Pr)2(N3)2]: synthesis of .alpha.-amino acids, *J. Org. Chem.*, **1988**, 53: 5185-5187.

38. March, J.; "Advanced Organic Chemistry: Reactions and Mechanisms", Wiley, **1999**, page 428 and 828, and references cited therein.

39. Carlsen P.H.J., Katsuki T., Martin V.S., Sharpless K.B., A greatly improved procedure for ruthenium tetroxide catalyzed oxidations of organic compounds, *J. Org. Chem.*, **1981**, 46: 3936-3938.

40. Trost, B. M., Comprehensive Organic Synthesis, Pergamon, **1991**, vol. 7, Section 3.2 (page 389-436) and Section 5.3 (page 703-716) and references cited therein.

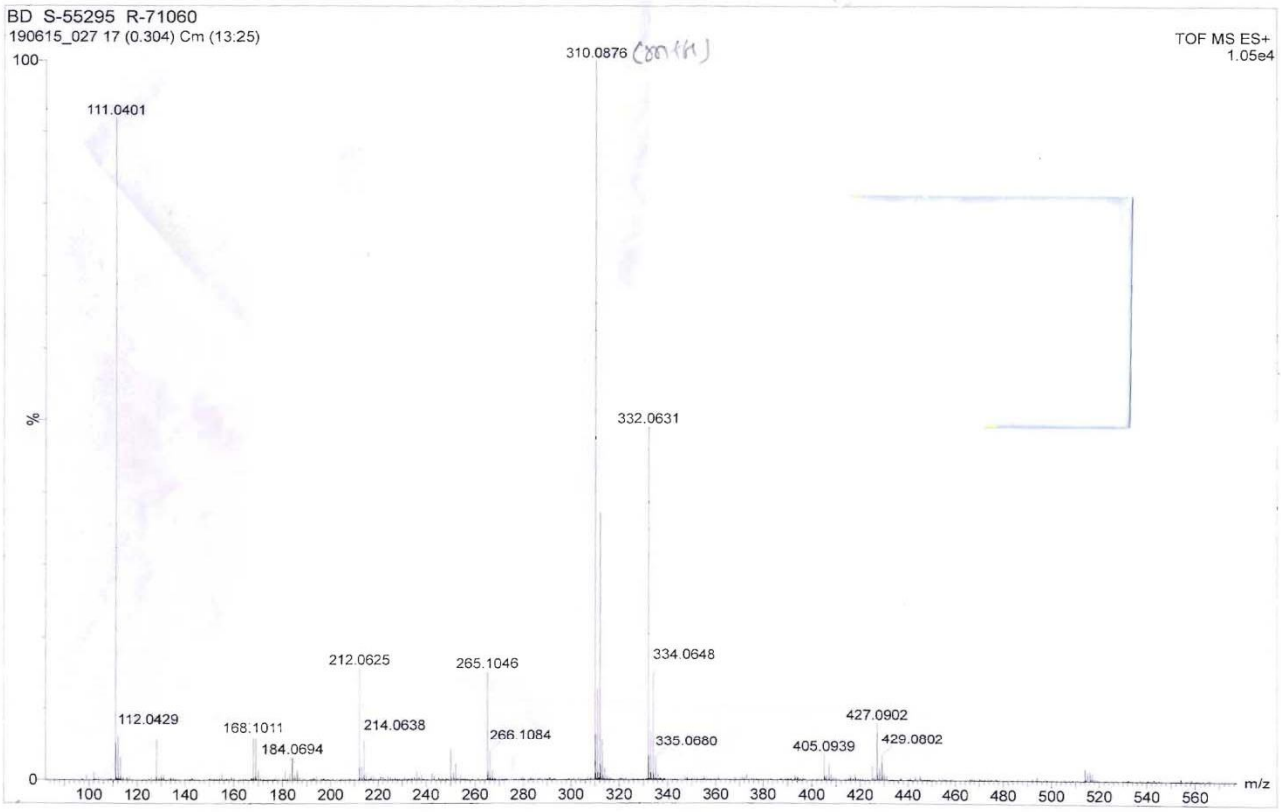
41. Paquette L.A., "Encyclopedia of reagents for Organic Synthesis", John Wiley & Sons, Inc, **1995**, 7: 4613-4616.

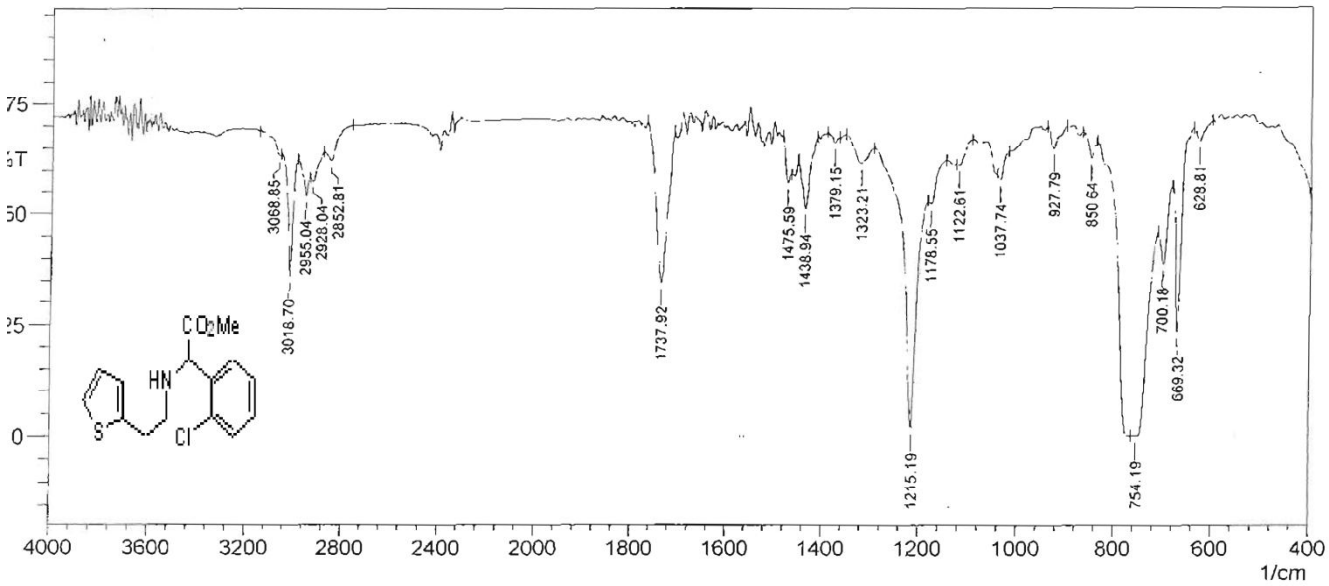
42. Sheldon R.A., "Chirotechnology", Marcel Dekker, Inc. NY, Basel, **1993**, 173-204.

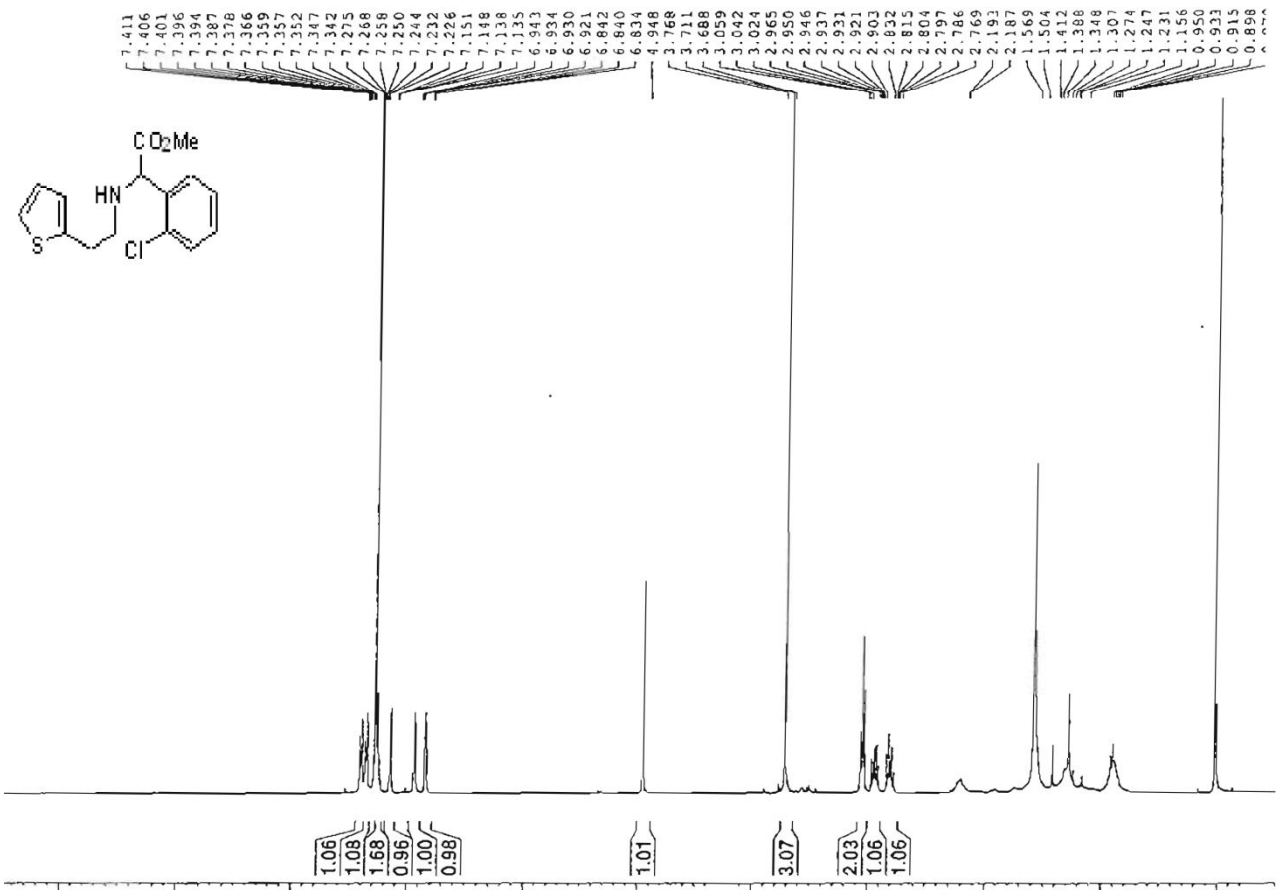
43. Sugidachi A., Ogawa T.,Kurihara A., Hagihara K., Jakubowski J.A., Hashimoto, M., Niitsu Y., Asai F., The greater *in vivo* antiplatelet effects of prasugrel as compared to clopidogrel reflect more efficient generation of its active metabolite with similar antiplatelet activity to that of clopidogrel's active metabolite, *J. Thromb. Haemost.*, **2007**, 5: 1545-1551.

Characterization Data

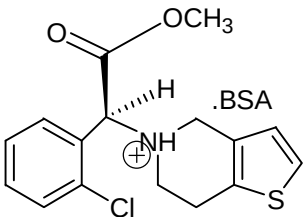
Intermediate 8

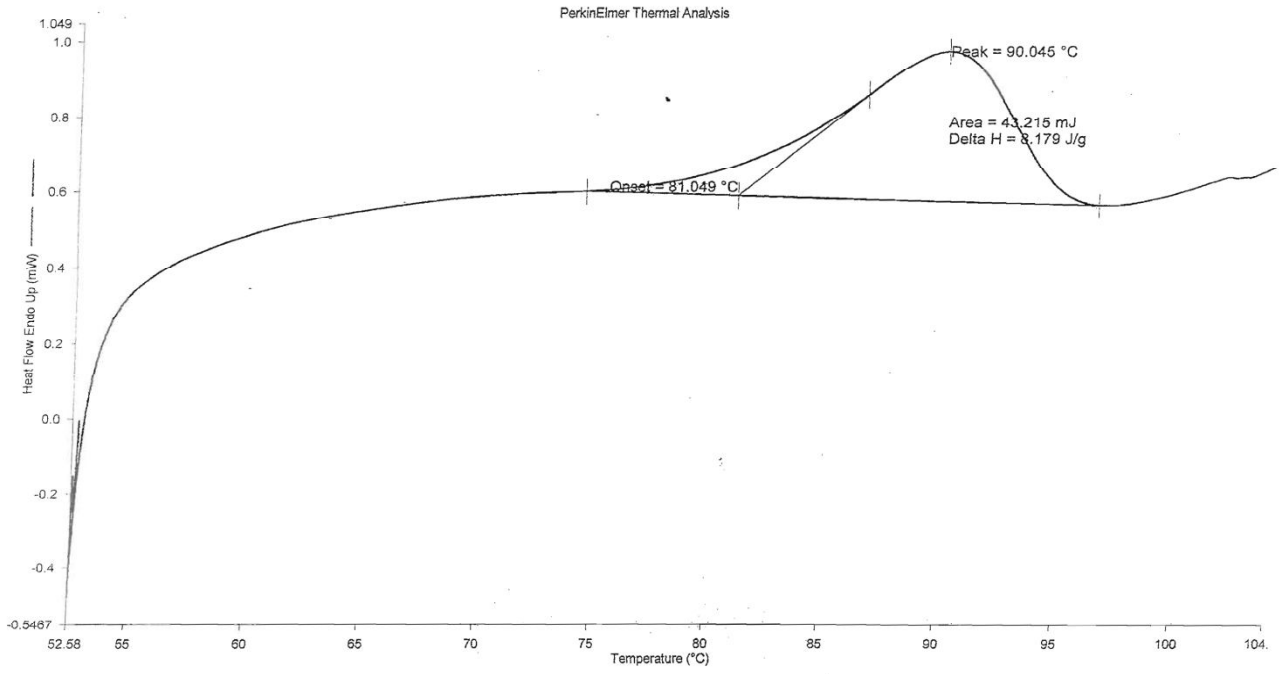

BD S-55295 R-71060
190615_027 17 (0.304) Cm (13:25)
TOF MS ES+
1.05e4
100
%
0
310.0876
111.0401
332.0631
334.0648
212.0625
265.1046
112.0429
168.1011
214.0638
184.0694
266.1084
335.0680
405.0939
427.0902
429.0802
m/z
100 120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560

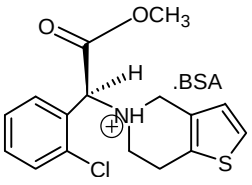

75
%T
50
25
0
3068.85
3018.70
2955.04
2928.04
2852.81
1737.92
1475.59
1438.94
1379.15
1323.21
1215.19
1178.55
1122.61
1037.74
927.79
850.64
754.19
700.18
669.32
628.81
CO2Me
HN
S
Cl
4000 3600 3200 2800 2400 2000 1800 1600 1400 1200 1000 800 600 400
1/cm

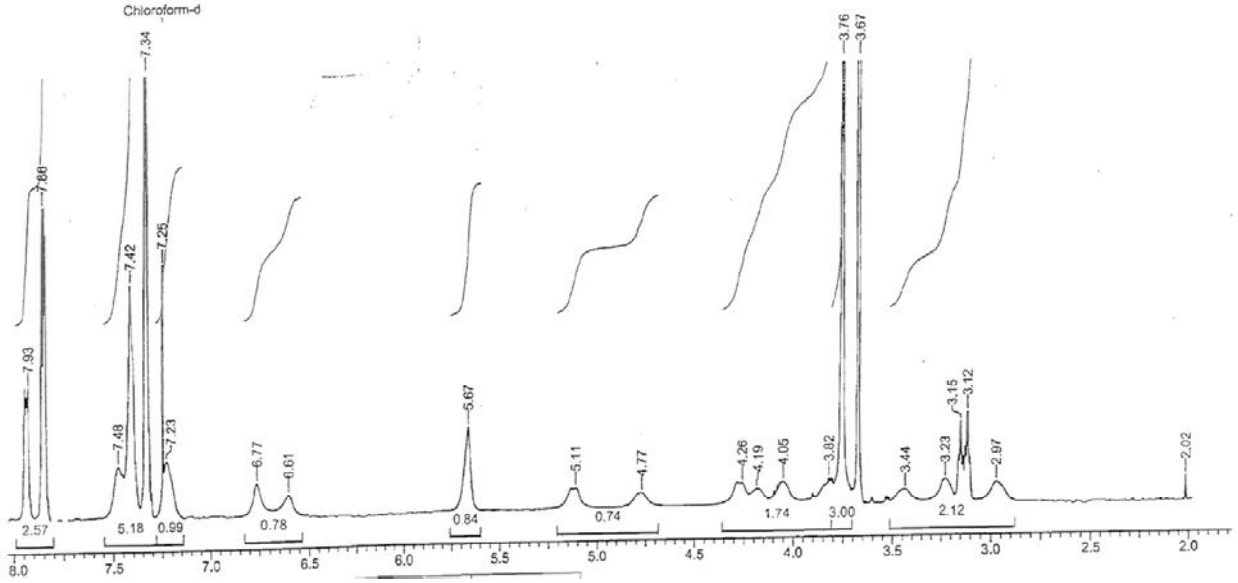

CO2Me
HN
S
Cl
7.411
7.406
7.401
7.396
7.394
7.387
7.378
7.366
7.359
7.357
7.352
7.347
7.342
7.275
7.268
7.258
7.250
7.244
7.232
7.226
7.151
7.148
7.138
7.135
6.943
6.934
6.930
6.921
6.842
6.840
6.834
4.948
3.768
3.711
3.688
3.059
3.042
3.024
2.965
2.950
2.946
2.937
2.931
2.921
2.903
2.832
2.815
2.804
2.797
2.786
2.769
2.187
1.569
1.504
1.412
1.388
1.348
1.307
1.274
1.247
1.231
1.156
0.950
0.933
0.915
0.898
1.06
1.08
1.68
0.96
1.00
0.98
1.01
3.07
2.03
1.06
1.06

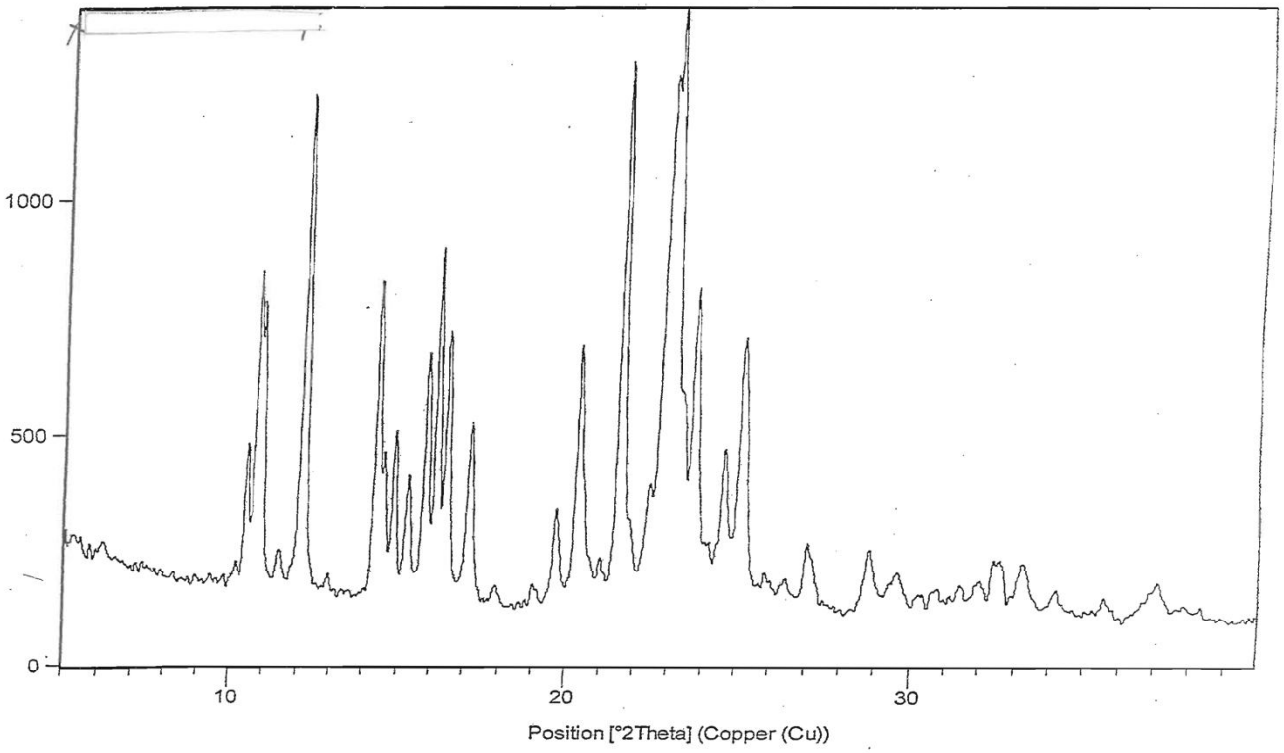
Toluene solvate of the benzenesulfonate salt of compound (1a)


O
OCH3
H
.BSA
NH
Cl
S

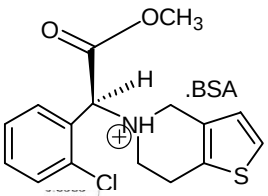

PerkinElmer Thermal Analysis
Peak = 90.045 °C
Area = 41.215 mJ
Delta H = 8.179 J/g
Onset = 81.049 °C
Heat Flow Endo Up (mW)
Temperature (°C)
1.049
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.5467
52.58
55
60
65
70
75
80
85
90
95
100
104.

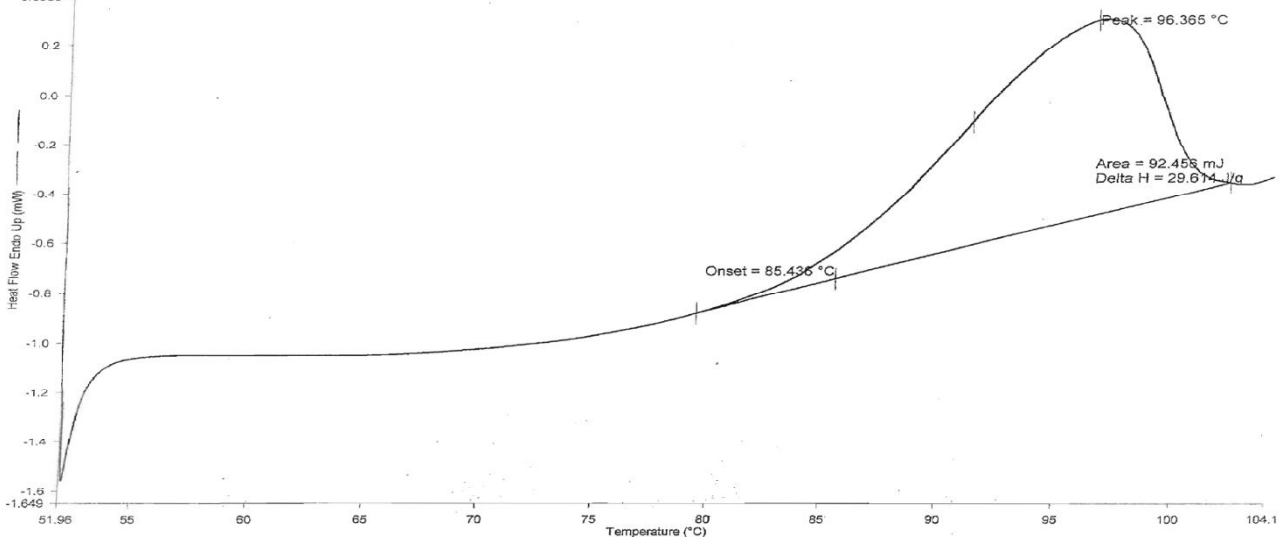

O
OCH3
H
.BSA
NH
Cl
S

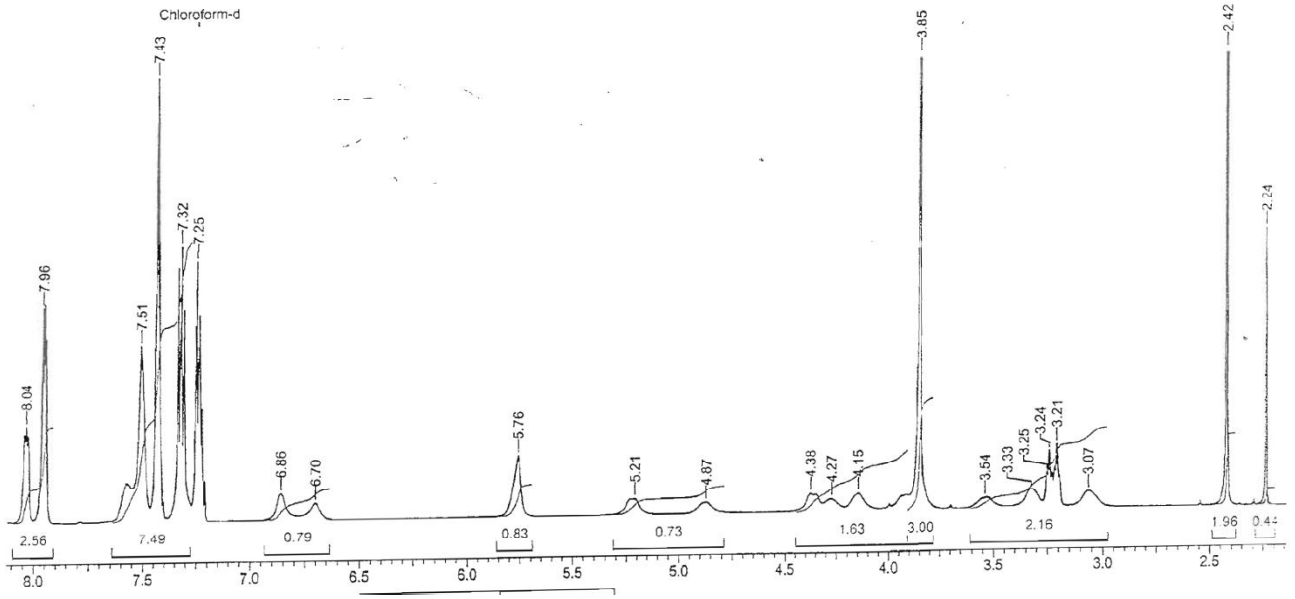

Chloroform-d
7.93
7.88
7.48
7.42
7.34
7.25
7.23
6.77
6.61
5.67
5.11
4.77
4.26
4.19
4.05
3.82
3.76
3.67
3.44
3.23
3.15
3.12
2.97
2.02
2.57
5.18
0.99
0.78
0.84
0.74
1.74
3.00
2.12
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0

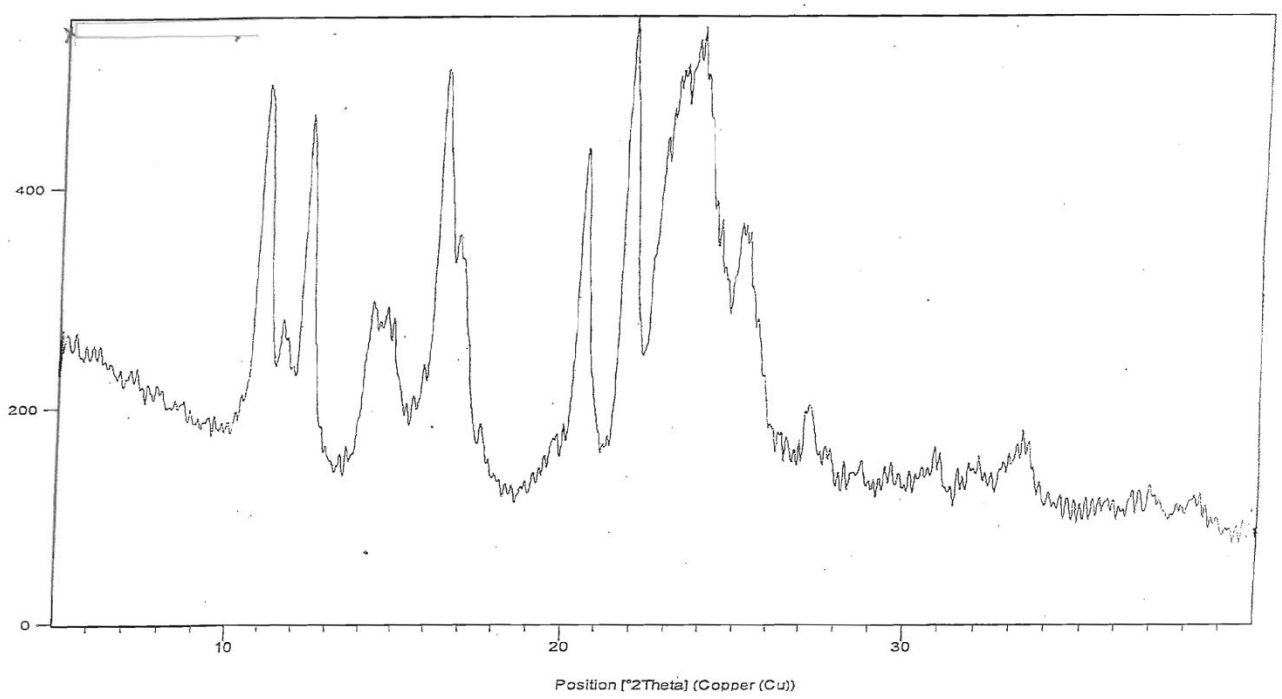

1000
500
0
10
20
30
Position [°2Theta] (Copper (Cu))

Dioxane solvate of the benzenesulfonate salt of compound (1a)

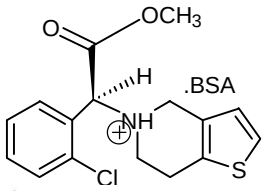

O
OCH3
H
.BSA
NH
Cl
S

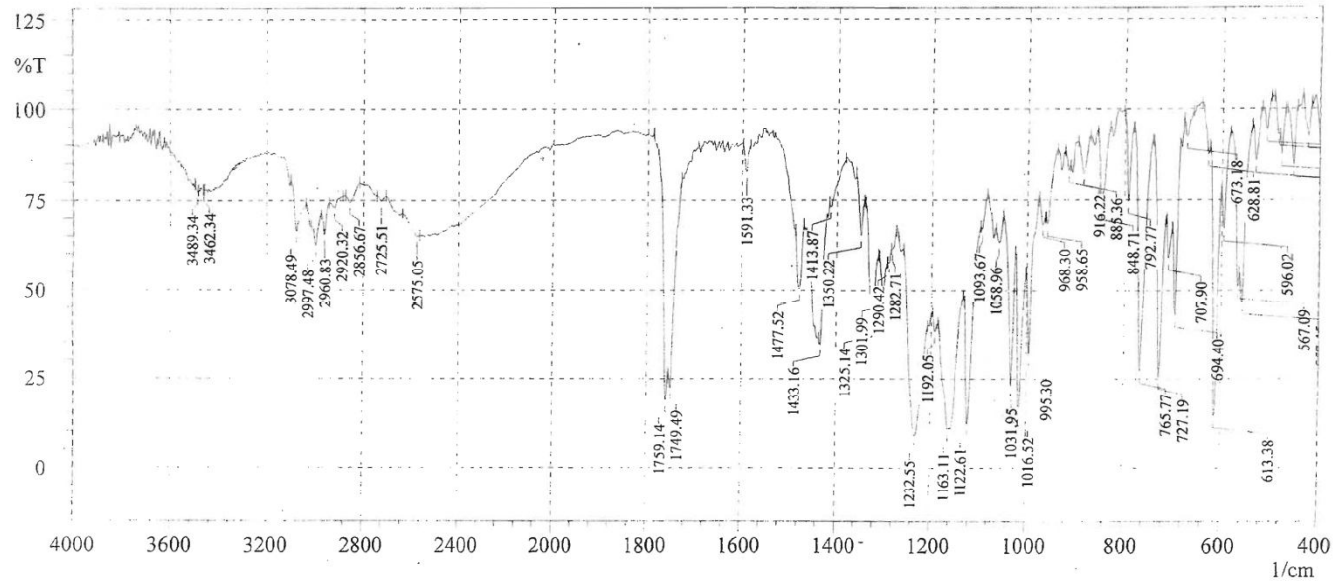

Peak = 96.365 °C
Area = 92.456 mJ
Delta H = 29.614 J/g
Onset = 85.436 °C
Heat Flow Endo Up (mW)
Temperature (°C)
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
-1.4
-1.649
51.96
55
60
65
70
75
80
85
90
95
100
104.1

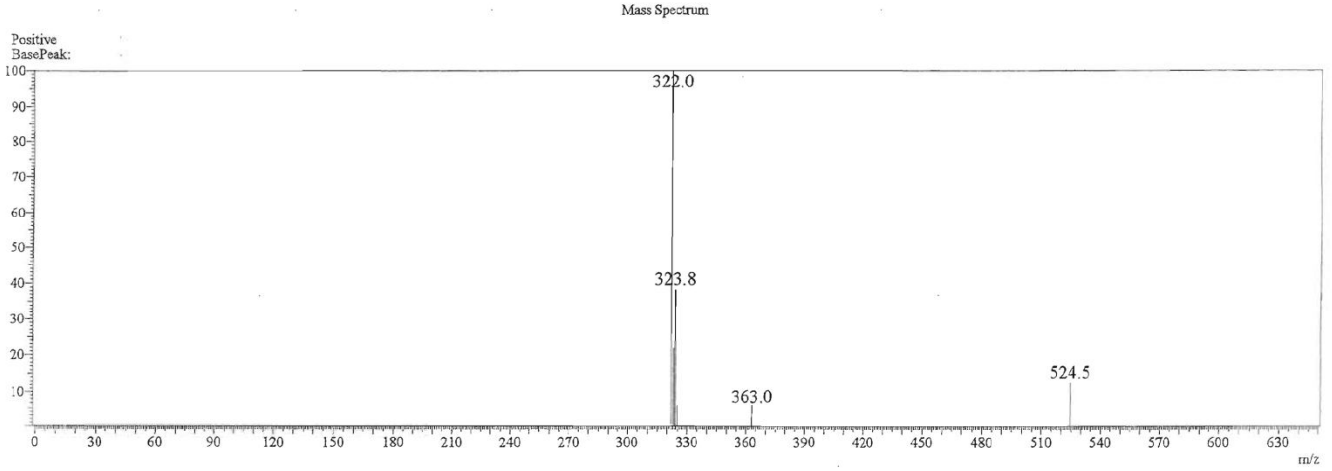

Chloroform-d
8.04
7.96
7.51
7.43
7.32
7.25
6.86
6.70
5.76
5.21
4.87
4.38
4.27
4.15
3.85
3.54
3.33
3.25
3.24
3.21
3.07
2.42
2.24
2.56
7.49
0.79
0.83
0.73
1.63
3.00
2.16
1.96
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5

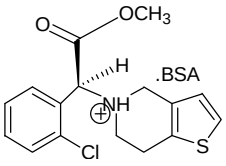

400
200
0
10
20
30
Position [°2Theta] (Copper (Cu))

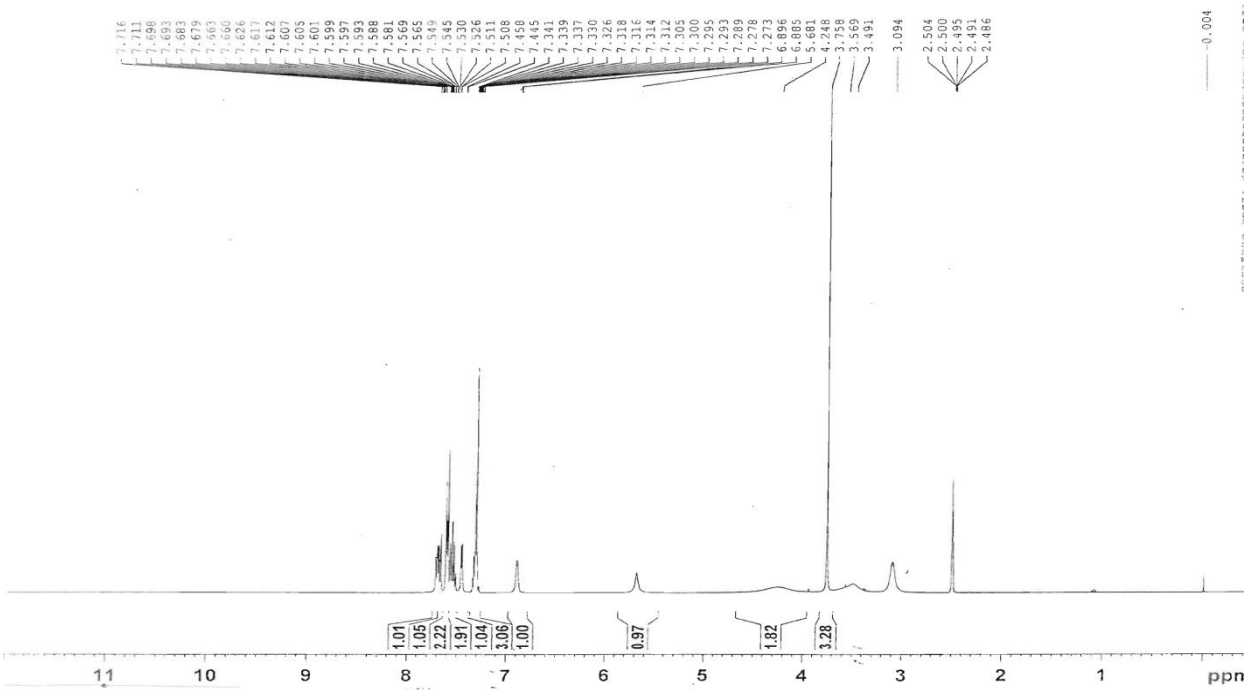
Unsolvated benzenesulfonate salt of compound (1a)

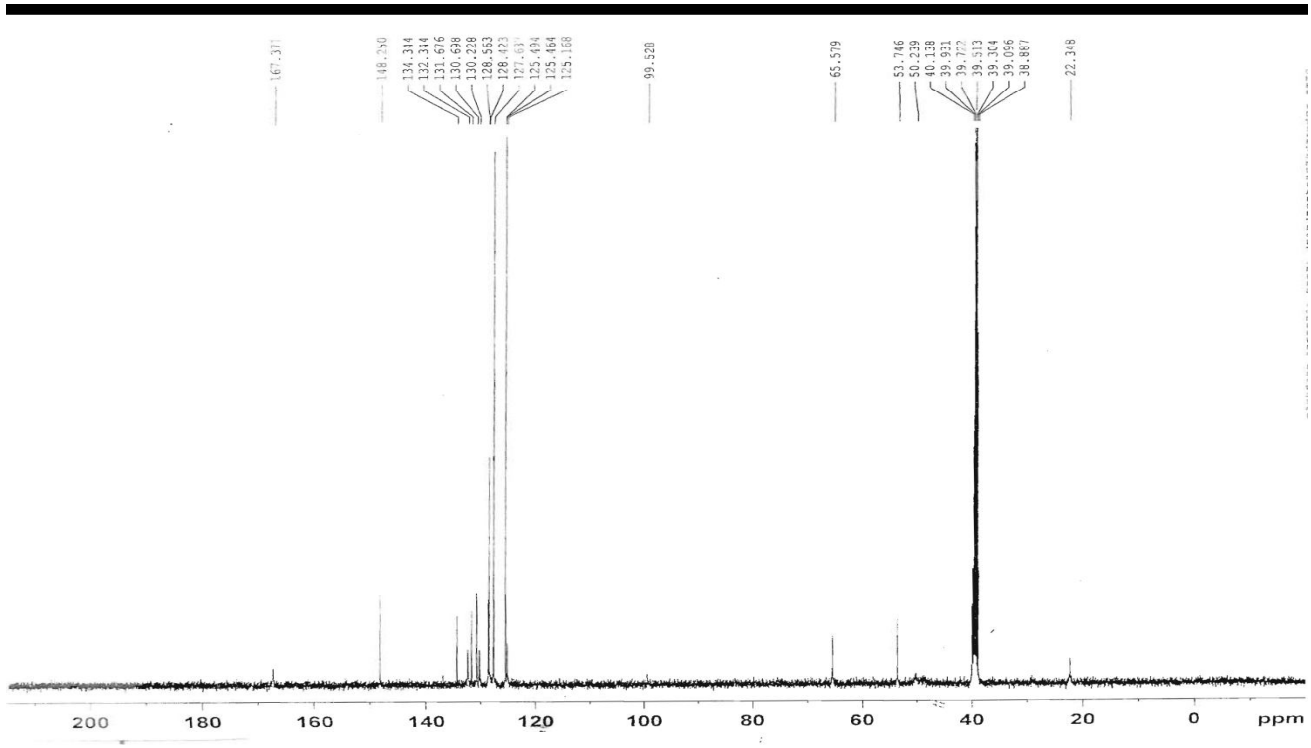

O
OCH3
H
.BSA
NH
Cl
S

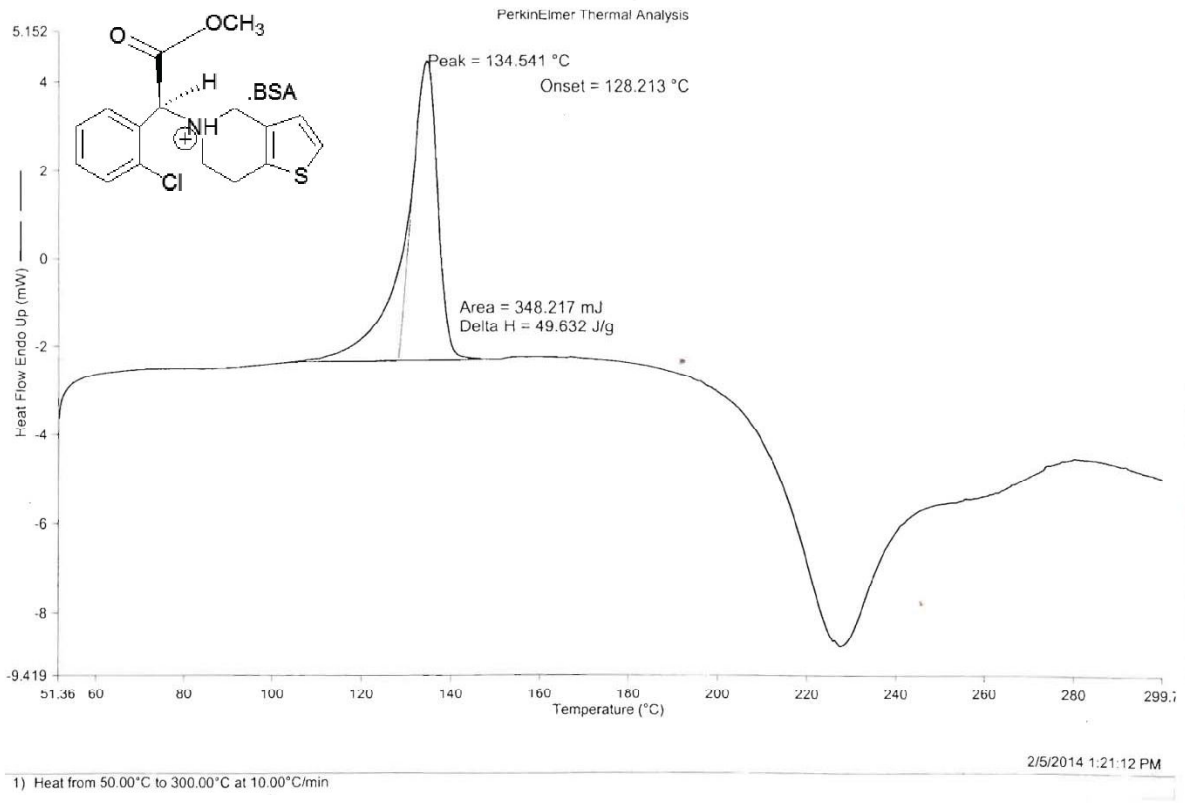

125
%T
100
75
50
25
0
3489.34
3462.34
3078.49
2997.48
2960.83
2920.32
2856.67
2725.51
2575.05
1759.14
1749.49
1591.33
1477.52
1433.16
1413.87
1350.22
1325.14
1301.99
1290.42
1282.71
1232.55
1192.05
1163.11
1122.61
1093.67
1058.96
1031.95
1016.52
995.30
968.30
958.65
916.22
885.36
848.71
792.77
765.77
727.19
705.90
694.40
673.18
628.81
613.38
596.02
567.09
4000
3600
3200
2800
2400
2000
1800
1600
1400
1200
1000
800
600
400
1/cm

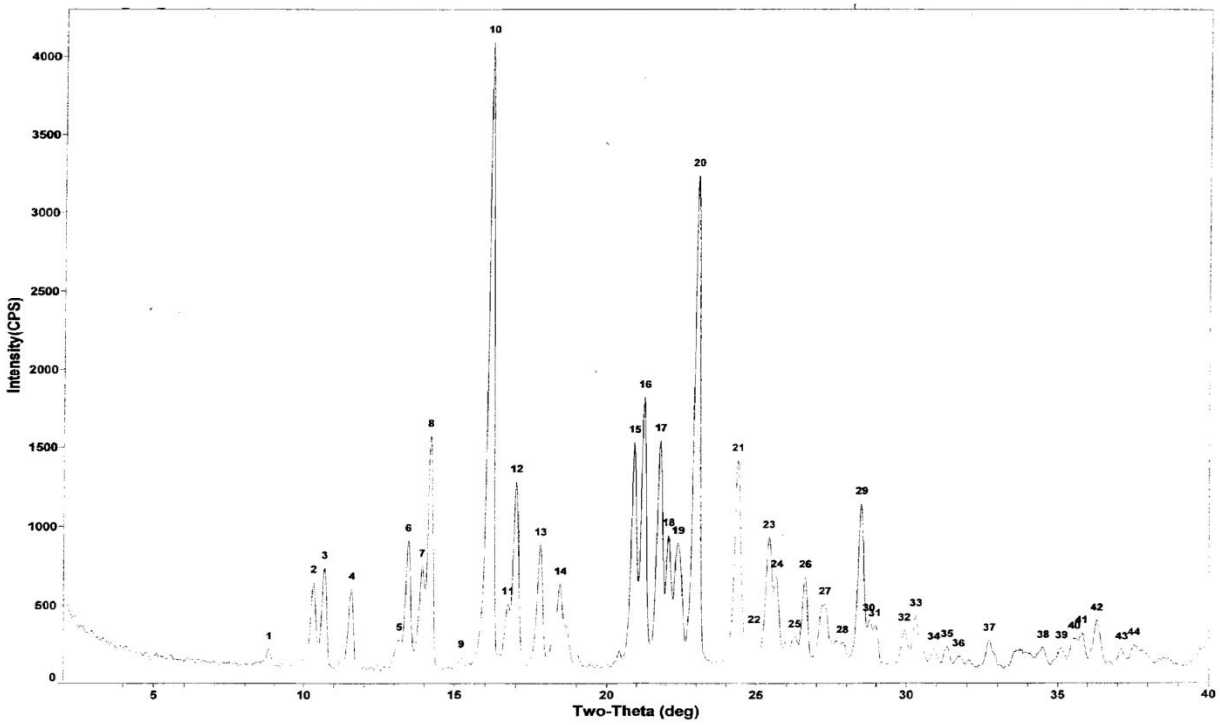

Mass Spectrum
Positive
BasePeak:
100
90
80
70
60
50
40
30
20
10
322.0
323.8
363.0
524.5
0
30
60
90
120
150
180
210
240
270
300
330
360
390
420
450
480
510
540
570
600
630
m/z


O
OCH3
H
.BSA
NH
Cl
S


7.716
7.711
7.698
7.693
7.683
7.679
7.663
7.660
7.626
7.617
7.612
7.607
7.605
7.601
7.599
7.597
7.593
7.588
7.581
7.569
7.565
7.549
7.545
7.530
7.526
7.511
7.508
7.458
7.445
7.341
7.339
7.337
7.330
7.326
7.318
7.316
7.314
7.312
7.305
7.300
7.295
7.293
7.289
7.278
7.273
6.896
6.885
5.681
4.248
3.758
3.569
3.491
3.094
2.504
2.500
2.495
2.491
2.486
0.004
1.01
1.05
2.22
1.91
1.04
3.06
1.00
0.97
1.82
3.28
11
10
9
8
7
6
5
4
3
2
1
ppn


167.371
148.230
134.314
132.314
131.676
130.698
130.228
128.563
128.423
127.681
125.494
125.464
125.168
99.528
65.579
53.746
50.239
40.138
39.921
39.722
39.513
39.304
39.096
38.887
22.348
200
180
160
140
120
100
80
60
40
20
0
ppm


PerkinElmer Thermal Analysis
Peak = 134.541 °C
Onset = 128.213 °C
Area = 348.217 mJ
Delta H = 49.632 J/g
Heat Flow Endo Up (mW)
Temperature (°C)
5.152
4
2
0
-2
-4
-6
-8
-9.419
51.36
60
80
100
120
140
160
180
200
220
240
260
280
299.7
2/5/2014 1:21:12 PM
1) Heat from 50.00°C to 300.00°C at 10.00°C/min


Intensity(CPS)
Two-Theta (deg)
4000
3500
3000
2500
2000
1500
1000
500
0
5
10
15
20
25
30
35
40