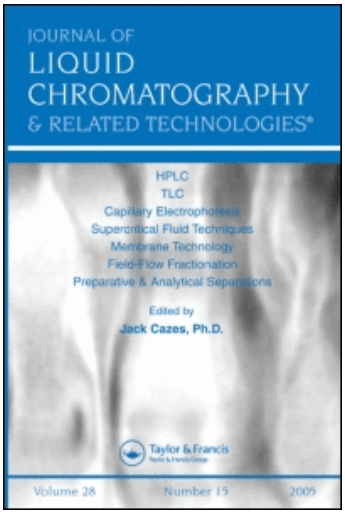

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USING NPS CHROMATOGRAPHY TO MONITOR MESYLATION REACTIONS

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ABSTRACT

Using nonporous silica (NPS) chromatography, mesylation reactions with unhindered and hindered amines were studied as proton scavengers. During reaction monitoring it is important to conduct sensitive and fast analyses. To optimally control the reaction quick feedback is necessary. The NPS proved to be highly suitable. When unhindered amines are used to remove the proton, the S_N2 mesylate reaction was observed to take place, followed by the S_N1 reaction to form the quaternary salt. The S_N1 reaction did not occur when the sterically hindered dicyclohexylamine was used. Liquid chromatography/mass spectrometry confirmed this process and was used to identify the final products when various amines were used as proton scavengers. This study confirmed that dicyclohexylamine is the preferred proton scavenger if one desires to obtain a stoichiometric amount of mesylate.

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INTRODUCTION

During the last few years nonporous silica (NPS) gels with a size of 1–2 μm were developed. The specific surface of the NPS gels decreases with the size of the particles. The smaller the silica gel particles are, the more efficient the separation. Because of the higher packing density, there is a fast mass transfer between the mobile and stationary phases, which leads to higher column efficiency. It is possible to work at higher pressures and higher flow rates due to the mechanical stability of the particle. This results in a decrease in analysis time (1,2). It is important to have quick feedback as well as sensitive and fast analysis for reaction monitoring. NPS has been proven to be highly suitable for this application (3,4).

During the synthesis of 3,5-dibenzyloxy benzyl alcohol (G1OH) to form higher generation dendrimers, a mesylated precursor was chosen because the sulfonate ion is an excellent leaving group (Fig. 1) (5). The alkyl sulfonate provides an indirect method for carrying out nucleophilic substitution on the G1OH. For the S_N2 reaction, the alcohol was first converted to the alkyl sulfonate. However, when an unhindered amine, for example, triethylamine, was used as a proton

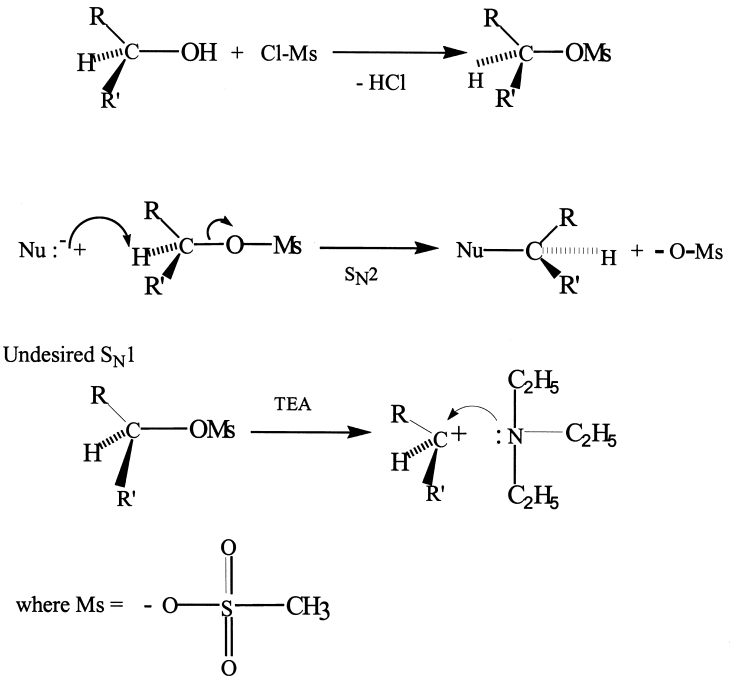


Figure 1. S_N2 mesylation reaction scheme.

scavenger to initiate the mesylation reaction, the reaction proceeded to the S_N1 product to form the undesired quaternary salt.

High-performance liquid chromatography (HPLC) and positive ion electrospray ionization mass spectrometry were used to monitor the mesylation reaction. During the chromatography of the 3,5-dibenzyloxy benzyl methyl sulfonate (G1Ms) product, the G1Ms peak area decreased over a short time to form the quaternary salt, which was identified by electrospray ionization (ESI) liquid chromatography (LC)/mass spectrometry (MS) for the amines investigated.

EXPERIMENTAL

LC Instrumentation

The HPLC instrumentation consisted of a Hewlett Packard model 1050 quaternary gradient pump with solvent degasser, Hewlett Packard model 1050 autosampler, Hewlett Packard model 1050 diode array detector (Chemical Analysis Group, Hewlett Packard, Waldbronn, Germany), and IBM personal computer PL 300 with Hewlett Packard ChemStation version 6.04 (Revision A) software.

MS Instrumentation

ESI was performed in the positive mode with nitrogen gas flow of 440 L/h. The response was optimum with a 3.50 kV capillary voltage, a source voltage of 19 V, and source temperature of 180°C. The instrument was operated at unit resolution in the selected ion monitoring mode, scanning from mass 100 to 1000. The Navigator liquid chromatograph/mass spectrometer was interfaced with a computer workstation running ThermoQuest's Excaliber 1.1 software.

Columns

Four analytical columns were used in this study. The first column was a Micra NPS RP18, 33 × 4.6 mm, stainless steel column packed with C_{18} 1.5-μm particle size NPS purchased from Eichrom Industries, Inc. (Darien, IL, USA). The second column used in this study was a Supelcosil ABZ, 75 × 4.6 mm, stainless steel column packed with C_{18} 3-μm particle size and 120-Å pore size silica, purchased from Supelco (Bellefonte, PA, USA).

The third and fourth columns were Luna C_{18} (2), 150 × 4.6 and 250 × 4.6 mm, stainless steel columns packed with 5-μm particle size and 100-Å pore size, purchased from Phenomenex (Torrance, CA, USA).

Chromatographic Procedure

The signal was scanned from 200 to 350 nm to record ultraviolet (UV) spectra, and the UV detection was at 210 nm. The mobile phase solvents were water (mobile phase A) and acetonitrile (mobile phase B), each containing 0.05% trifluoroacetic acid. The mobile phase compositions used for each of the columns were A/B 40:60 for the NPS column, A/B 50:50 for the 150 and 250 mm Luna columns, and A/B 65:35 for the Supelco ABZ+ column. The flow rate was 1 mL/min with an injection volume of 1 μL for the conventional columns and 0.2 μL injection volume for the NPS column.

Chemicals

Benzyl alcohol, triethylamine (TEA), dicyclohexylamine (DCX), pyridine, dimethylaminopyridine, and di-isopropyl ethyl amine were purchased from Aldrich (Milwaukee, WI, USA). The chemicals, whose purity is listed by Aldrich, were used, as is, without further purification. Trifluoroacetic acid, anhydrous, protein sequencing grade, was purchased from Sigma (St. Louis, MO, USA). Burdick and Jackson high-purity solvent, acetonitrile UV grade was used (AlliedSignal, Morristown, NJ, USA). Water was purified with the Milli-Q Water System (Millipore, Bedford, MA, USA).

Sample Preparation

A 0.1-mL aliquot was withdrawn from the reaction mixture using an 18-gauge, 6-inch hypodermic needle attached to a 1-mL glass syringe (Becton, Dickinson & Co., Franklin Lakes, NJ, USA). The solution was diluted to 2 mL with a 1:1 mixture of acetonitrile/water. The sample solution was filtered through an Acrodisc 13CR (PFTE), 13 mm, 0.2-μm pore size syringe filter, purchased from Pall Gelman Laboratory (Ann Arbor, MI, USA), and transferred into a HPLC vial.

Mesylation Reaction

A solution of 1.0 equivalent of G1OH in 100 mL of anhydrous toluene was stirred under N_2. The reaction flask was then cooled to 0°C in an ice bath. Dicyclohexylamine (or the proton scavenger being investigated), 1.1 equivalents, was added slowly to the cold mixture through a 5-mL glass syringe. While main-

taining the solution below 8°C, methyl sulfhonyl chloride, 2.02 equivalents, was added, dropwise, to the mixture using the 5-mL glass syringe.

It is very important to maintain the temperature below 8°C to obtain a high yield and good purity. When the addition was completed, the solution was allowed to continue to stir for 1 h. Upon completion of the reaction, 200 mL of methylene chloride was added to the mixture, and the mixture was filtered. The precipitate was washed with methylene chloride. The collected filtrate was washed with 100 mL of double-distilled water. The organic layer was collected.

The aqueous layer was further extracted with methylene chloride twice (2 $\times$100 mL). The combined organic layers were then washed with 100 mL of 0.5 *N* HCl, 100 mL of 5% $NaHCO_3$, and saturated NaCl and then dried over $MgSO_4$. The solution was filtered, and the solvent was removed in vacuo. Pale yellow crystals, 4.01 g, were obtained, which were stored under refrigeration.

In the Fourier transform infrared spectrum (KBr pellet), absorptions at 3065 cm^{-1} (Ar C–H, st), 1454 cm^{-1} (CH_3, as st), 1379 cm^{-1} (SO_2, st, antisym with overlap of CH_3), 1275 cm^{-1} (C–O–C, as st), 1171 cm^{-1}, (SO_2, sym st) with combination bands of 755 and 703 cm^{-1}, which are diagnostic for a monosubstituted aromatic ring, out of plane bending vibration, were recorded The ^{1}H-NMR spectrum ($CDCl_3$, trimethylsilane) shows δ 7.41 (m, Ar_1H, 10H), 7.25 (m, Ar_0H, C_4–H, 1H), 6.634 (s, Ar_0H, $C_{2,5}$–H, 2H), 5.148 (s, CH_2–SO_3–CH_3, 2H), 5.043 (s,

Table 1. Comparison of the Amounts of Desired G1Ms and Undesired Product, G1 Amine, when Different Proton Scavengers Are Used in the Mesyalation Reaction[a]

		Area (%)		
Proton Scavenger	Time (h)	G1Ms		G1Amine
Triethylamine	0	None		11
	0.5	None		12
	1.0	None		22
	3.0	None		23
Dicyclohexylamine	0.5	94.0	98.6[b]	None
	1.0	96.0	99.0	None
	1.5	96.0	—	None
	3.5	99.0		None
Dimethylamino-	3.5	15.0		—
Pyridine	14.0	30.0		4.0
	48.0	35.0		11.0

[a]Reaction monitored using NPS chromatography.
[b]Optimized reaction conditions.

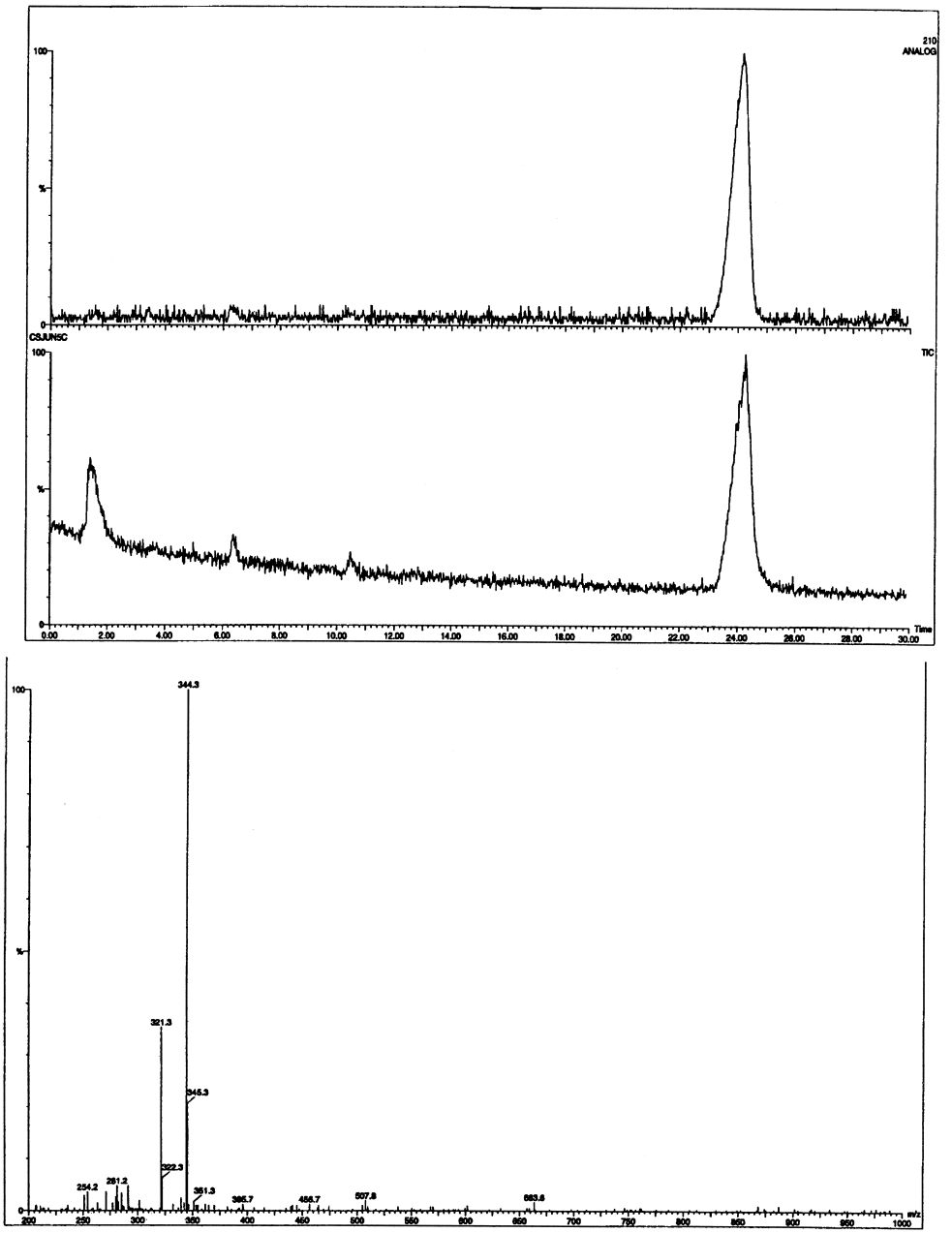


Figure 2. Total ion chromatogram, analog response, and G1OH mass spectrum. Mesylation reaction using TEA as the proton scavenger.

Ar_1–CH_2–O, 4H), and 2.83 (s,–SO_3–CH_3, 3H). The ESI mass spectrum confirmed the G1Ms mass with the $[M + H]^+$, 399 and the sodium adduct ion $[M + Na]^+$, 421.

RESULTS AND DISCUSSION

Initial mesylation reactions were monitored with a conventional 25 cm × 4.6 mm HPLC column. These experiments included the unhindered amine, TEA, as the proton scavenger. The major product was found to be the starting alcohol, G1OH, and a significant amount of the S_N1 reaction product, the undesired amine (Fig. 1 and Tab. 1). When TEA was used as the proton scavenger, the quaternary amine, $[M + H]^+$ 404 , was formed, but due to steric hindrance only the 3,5-dibenzyloxy benzyl di-isopropyl amine was observed, $[M + H]^+$ 404 and not *m z* 453 when di-isopropyl ethyl amine was used as a proton scavenger (Fig. 2).

Table 2. Retention Times Observed for the Mesylation Reactants and Products Using the Proton Scavengers DCX and TEA

	Time (min)	
Compound	DCX	TEA
NPS (40:60)		
Toluene	0.28	0.28
G1Ms	0.93	—
G1OH	2.50	—
G1TEA		1.3
Supelco ABZ+ (65:35)		
Toluene	0.79	0.79
G1Ms	8.59	—
G1OH	5.14	5.08
G1TEA		8.43
15-cm Luna (50:50)		
G1Ms	1.78	1.78
G1OH	9.60	
G1TEA	4.20	4.20
25-cm Luna (50:50)		
Toluene	3.24	3.24
G1Ms	10.52	—
G1OH	6.51	6.51
G1TEA	—	24.0

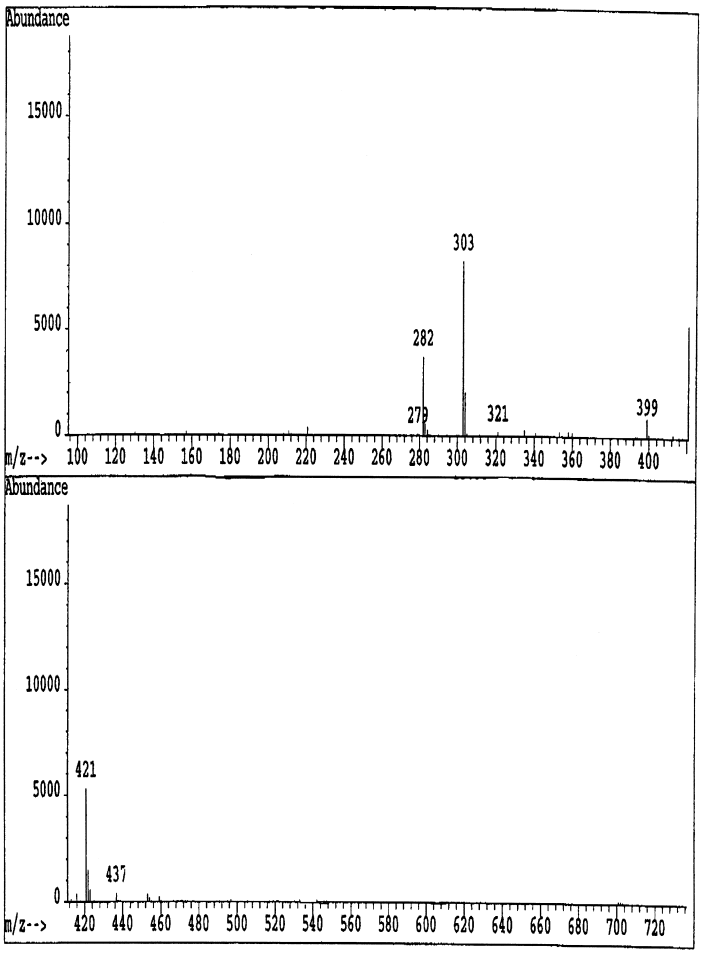


Figure 3. ESI mass spectrum of G1Ms

Because the mesylation reaction was found to be very fast, approximately 1 h, shorter length HPLC columns were investigated to monitor the reaction. The goal was to prevent the S_N1 reaction by using a proton scavenger, which would give the desired mesylate product. Although the Phenomenex 15-cm Luna and Supelco 7.5-cm ABZ+ columns gave shorter retention times (10 min) (Tab. 2), a faster method for reaction monitoring was desired. The 33-mm NPS column was ideal for monitoring the mesylation reaction with its total time of 3 min. Several proton scavengers were investigated to prevent the S_N1 reaction: pyridine, triethy-

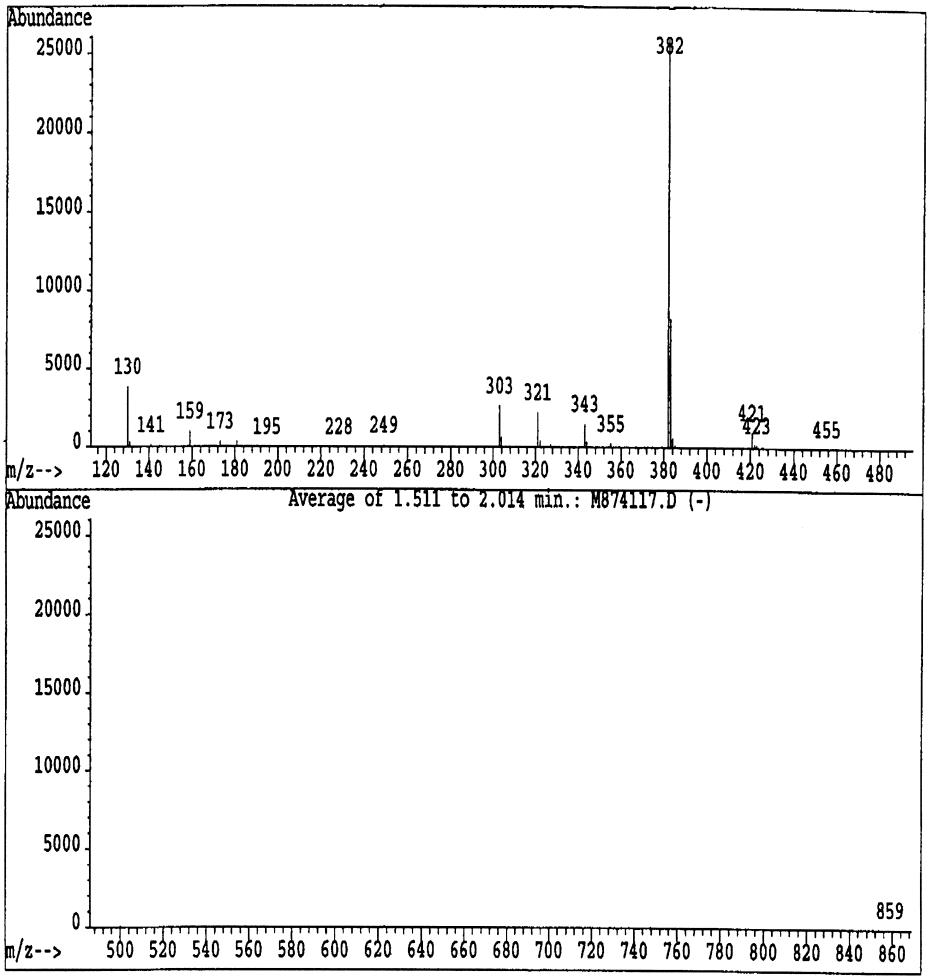


Figure 4. ESI mass spectrum of 3,5-dibenzyloxy benzyl pyridinium salt.

lamine, di-isopropyl ethyl amine, 4-dimethylamino-pyridine, and dicyclohexylamine. The structures of the S_N1 products are shown in Table 3, and their relative amounts are found in Table 1.

When DCX was used as the proton scavenger, the desired G1Ms was obtained with no detectable S_N1 product. The reaction was complete within 1 h, and NPS chromatography permitted quick feedback to optimally control the reaction.

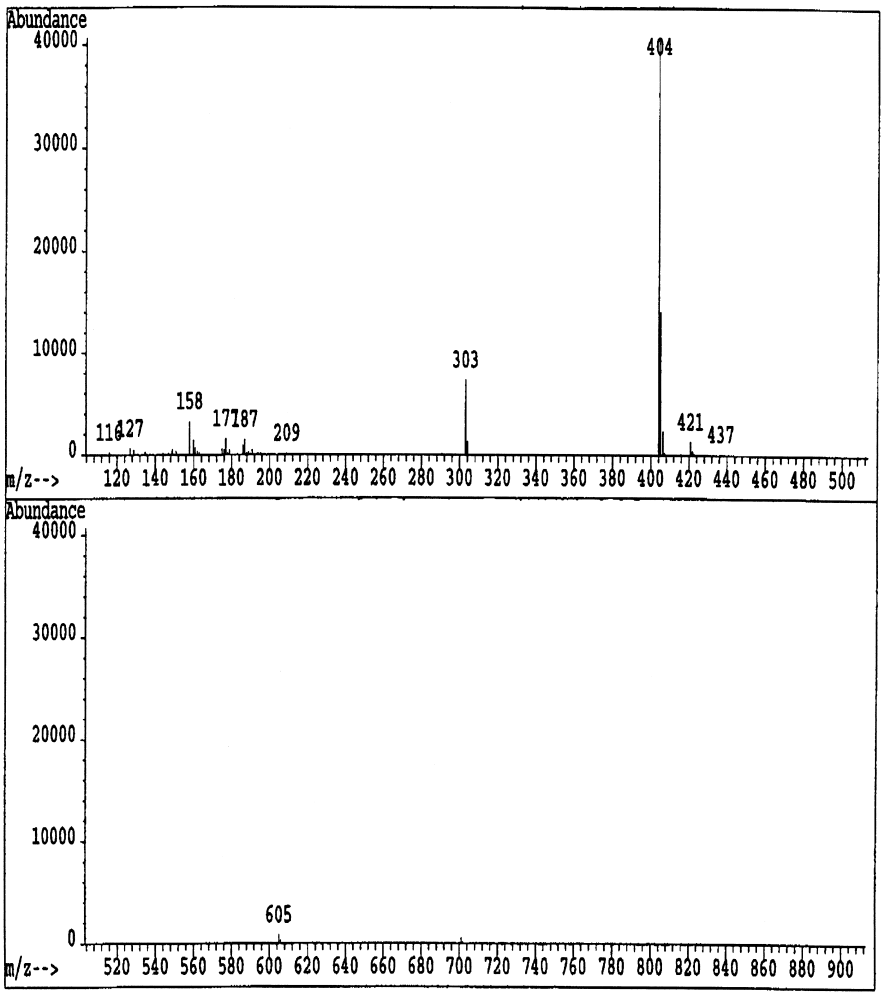
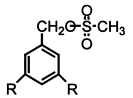
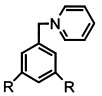
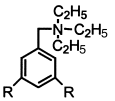
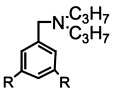
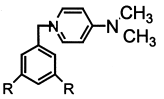
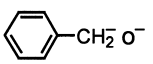


Figure 5. ESI mass spectrum of 3,5-dibenzyloxy benzyl triethylamine salt.

Table 3. Structure for the G1OH , G1Ms, and the Undesired S_N1 Amine Product with the Protonated Molecular Ion Observed

Structure[a]	Formula Weight	$[M + H]^+$	Sodium Adduct
	320	321	344
	398	399	421
	381	382	
	404	404	
	403	404	
	425	426	

[a]where R = 

CONCLUSION

NPS was ideal for monitoring the mesylation reaction of G1OH to the G1Ms. It provided rapid and sensitive high-performance liquid chromatography. When the hindered proton scavenger DCX was used, stoichiometric amounts of the desired mesylate were obtained. Additional experiments using various proton scavengers, alcohols, and mesylation reagents will be used in future studies.

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