

# Derivatization



Derivatization is primarily performed to modify an analyte's functionality to enable chromatographic separations. For more than 40 years, Regis has been a leader in the manufacture of highly pure derivatization reagents for gas chromatography. The formation of chemical derivatives to facilitate meaningful analysis has long been a common practice in gas chromatography. For the analytical chemist, judicious use of derivatization can be the key to unlocking and simplifying a great many complex and perplexing separation problems. Derivatization, accomplished through alteration of functional groups, provides:

- Increased sample volatility
- Improved selectivity and chromatographic efficiency
- Enhanced detectability

Additional procedures and references are available on our Web site at: [www.registech.com/gc](http://www.registech.com/gc).



# Gas Chromatography (GC) Derivatization

Sample volatility or thermal stability is crucial in GC applications. If a sample does not possess these important characteristics, GC analysis is highly unproductive.

Derivatization techniques have been developed to address these issues to insure successful separations. In GC Derivatization, replacement of active hydrogen in functional groups, such as -COOH, -OH, -NH, and -SH, is the primary area of concern and is accomplished through either silylation, acylation or alkylation.

## Silylation

Silylation is the most widely used derivatization procedure for sample analysis by GC. Silylation reagents are popular because they are easy to use and readily form derivatives. In silylation, an active hydrogen is replaced by an alkylsilyl group, such as trimethylsilyl (TMS) or *t*-butyl-dimethylsilyl (*t*-BDMS). Compared to their parent compounds, silyl derivatives are more volatile, less polar, and more thermally stable. As a result, GC separation is improved and detection is enhanced.

Silylation reagents are generally moisture sensitive, requiring them to be sealed under nitrogen to prevent deactivation. The derivatives of TMS reagents are also moisture sensitive. In response to this difficulty, *t*-BDMS reagents were introduced, which enabled the formation of derivatives 10,000 times more stable to hydrolysis than the TMS ethers.

Both TMS and *t*-BDMS reagents are suitable for a wide variety of compounds, offer excellent thermal stability and can be used in a variety of GC conditions and applications.

Analysis by the popular combination of gas chromatography and mass spectrometry (GS/MS) often requires special sample derivatization. Particularly effective in these applications is MTBSTFA.



## Acylation

Acylation reagents offer the same types of advantages available from silylation reagents: creating less polar, more volatile derivatives. However, in comparison to silylating reagents, the acylating reagents more readily target highly polar, multi-functional compounds, such as carbohydrates and amino acids. In addition, acylating reagents provide the distinct advantage of introducing electroncapturing groups, thus enhancing detectability during analysis.

Generally, these reagents are available as acid anhydrides, acyl derivatives, or acyl halides. The acyl halides and acyl derivatives are highly reactive and are suitable for use where steric hindrance may be a factor. Acid anhydrides are supplied in a number of fluorinated configurations, which improve detection. These fluorinated anhydride derivatives are used primarily for Electron Capture Detection (ECD), but can also be used for Flame Ionization Detection (FID). Fluorinated anhydrides are often used in derivatizing samples to confirm drugs of abuse. Despite the special utility of these reagents, their acidic nature requires that any excess or byproducts be removed prior to analysis to prevent deterioration of the column.





## Alkylation

As with other derivatization reagents, alkylation reagents reduce molecular polarity by replacing active hydrogens with an alkyl group. These reagents are used to modify compounds having acidic hydrogens, such as carboxylic acids and phenols. Alkylation reagents can be used alone to form esters, ethers, and amides—or they can be used in conjunction with acylation or silylation reagents. A two-step approach is commonly used in the derivatization of amino acids, where multiple functional groups on these compounds may necessitate protection during derivatization.

Due to the availability of reagents and their ease of use, esterification (the reaction of an acid with an alcohol in the presence of a catalyst to form an ester) is the most popular method of alkylation. Alkylation reagents are available in several configurations that enable the formation of a variety of esters. Alkyl esters are stable, and can be formed quickly and quantitatively. By altering the length of the substituted alkyl group, retention of the derivative can be varied. In addition to the formation of simple esters, alkylation reagents can be used in extractive procedures where biological matrices may be present.

## GC Chiral Derivatization

GC analysis of enantiomeric compounds on nonracemic or achiral stationary phases requires the use of enantiopure derivatization reagents. These reagents generally target one specific functional group to produce diastereomers of each of the enantiomeric analytes. From the resulting chromatograms, calculations are conducted to determine the enantiomeric concentration of the analyte.

## GC Derivatization Method

Functional Group	Silylation	Acylation	Alkylation
<b>Active Hydrogens</b>	BSTFA, BSTFA/TMCS, Deriva-Sil, Hydrox-Sil, TBH, MSTFA, MTBSTFA, TMSI	PFPOH/PFPA	DMF Dialkylacetals,
<b>Carboxylic Acids</b>	BSTFA, Hydrox-Sil Conc., MTBSTFA, TMSI	PFPOH/PFPA	BF <sub>3</sub> /Methanol, BF <sub>3</sub> /n-Butanol, DMF Dialkylacetals, TBH
<b>Alcohols and Phenols:</b> unhindered and moderately hindered	BSA, BSTFA/TMCS, HMDS, MTBSTFA/ <i>t</i> -BDMCS	HFBI, Fluorinated anhydrides (HFBA, PFPA, TFAA), MBTFA, MCF*	DMF Dialkylacetals, PFB-Br/TBA-H-SO <sub>4</sub> , TBH
<b>Alcohols and Phenols:</b> highly hindered	BSTFA/TMCS, Deriva-Sil, Deriva-Sil Conc.	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, PFBCI	DMF Dialkylacetals, PFB-Br/TBA-H-SO <sub>4</sub> , TBH
<b>Amines:</b> primary and secondary	BSTFA, MTBSTFA/ <i>t</i> -BDMCS	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, MBTFA, PFBCI, TPC*	DMF Dialkylacetals, TBH
<b>Amides</b>	BSA, BSTFA, BSTFA/TMCS, Deriva-Sil Conc.	HFBI	DMF Dialkylacetals,
<b>Amino Acids</b>	BSTFA, TMSI	HFBI (+ Silylation)	DMF Dialkylacetals, TBH 3N HCl in n-Butanol
<b>Catecholamines</b>	TMSI	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI	
<b>Carbohydrates and Sugars</b>	HMDS, Hydrox-Sil AQ, TMSI	MBTFA	
<b>Inorganic Anions</b>	BSTFA, MTBSTFA		
<b>Nitrosamines</b>		HFBA	
<b>Sulfonamides</b>	BSTFA	Fluorinated anhydrides (HFBA, PFPA, TFAA)	DMF Dialkylacetals, PFB-Br/TBA-H-SO <sub>4</sub>

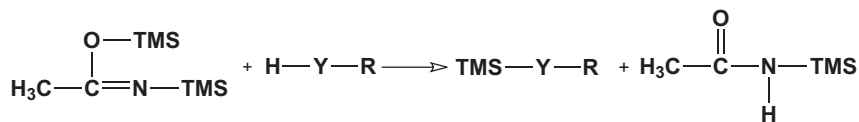
Derivatization reagents are listed in alphabetical order, not in order of preference.

\*For Chiral Analysis

Source: Knapp, D.R. Handbook of Analytical Derivatization Reactions; John Wiley and Sons: New York, 1979.

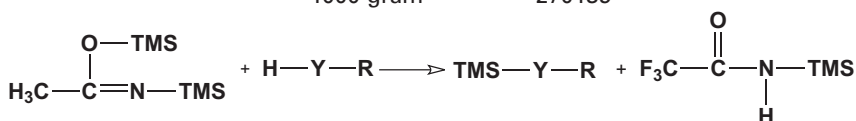
# Silylation Reagents

BSA	Product	Size	Catalog #
<b><i>N,O</i>-Bis(trimethylsilyl)acetamide</b> • Forms highly stable TMS derivatives with most organic functional groups under mild reaction conditions.	BSA	10 x 1 gram	270501
		4 x 5 gram	270502
		25 gram	270503
		100 gram	270504



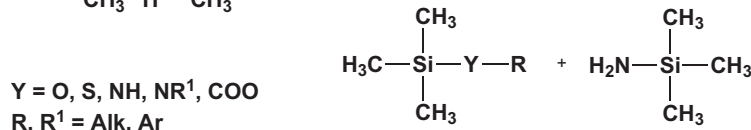
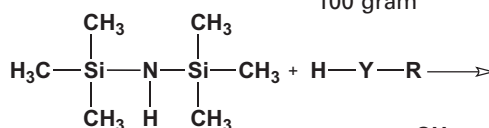
TMS = Si(CH<sub>3</sub>)<sub>3</sub>      Y = O, S, NH, NR<sup>1</sup>, COO  
R, R<sup>1</sup> = Alk, Ar

BSTFA-Regisil® BSTFA +TMCS (1%, 10%)	Product	Size	Catalog #
<b><i>N,O</i>-Bis(trimethylsilyl)trifluoroacetamide</b> • Reacts faster and more completely than BSA due to presence of trifluoroacetyl group. • The high volatility of BSTFA and its byproducts results in separation of early eluting peaks. • Highly volatile and stable products result in low detector noise and fouling. • Excellent solubility. • Addition of TMCS catalyzes reactions of hindered functional groups in secondary alcohols and amines.	Regisil® RC-1	10 x 1 gram	270111
	BSTFA	4 x 5 gram	270112
		25 gram	270113
		100 gram	270114
		1000 gram	270116
	Regisil® RC-2	10 x 1 gram	270121
	BSTFA +1% TMCS	4 x 5 gram	270122
		25 gram	270123
		100 gram	270124
		1000 gram	270126
	Regisil® RC-3	10 x 1 gram	270131
	BSTFA +10% TMCS	4 x 5 gram	270132
		25 gram	270133
		100 gram	270134
		1000 gram	270135



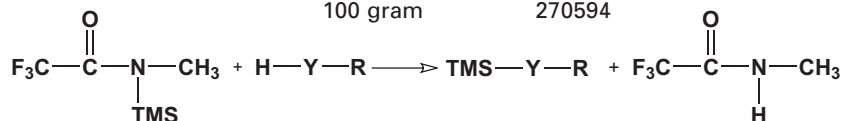
TMS = Si(CH<sub>3</sub>)<sub>3</sub>      Y = O, S, NH, NR<sup>1</sup>, COO  
R, R<sup>1</sup> = Alk, Ar

HMDS	Product	Size	Catalog #
<b>Hexamethyldisilazane</b> • Weak TMS donor, used for silylation of carbohydrates. • Used as mixture with pyridine and trifluoroacetic acid.	HMDS	25 gram	270651
		100 gram	270652



Y = O, S, NH, NR<sup>1</sup>, COO  
R, R<sup>1</sup> = Alk, Ar

MSTFA	Product	Size	Catalog #
<b><i>N</i>-Methyltrimethylsilyltrifluoroacetamide</b> • Most volatile of the TMS-acetamides. • Useful in the analysis of volatile trace materials.	MSTFA	10 x 1 gram	270590
		10 gram	270589
		25 gram	270593
		100 gram	270594



TMS = Si(CH<sub>3</sub>)<sub>3</sub>      Y = O, S, NH, NR<sup>1</sup>, COO  
R, R<sup>1</sup> = Alk, Ar

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# Silylation Reagents

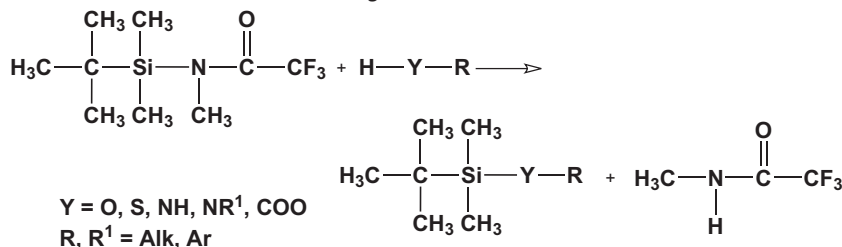
## MTBSTFA

### MTBSTFA + 1% *t*-BDMCS

*N*-Methyl-*N*-(*t*-butyldimethylsilyl)trifluoroacetamide

- Replaces active hydrogens to form *t*-BDMS derivatives.
- Derivatization is usually complete upon dissolution with this exceptionally strong, yet mild silylating reagent.
- MTBSTFA derivatives are 104 times more stable to hydrolysis than their corresponding TMS derivatives.
- Produces easily interpreted mass spectra for GC/MS.
- Addition of *t*-BDMCS catalyzes reactions of hindered alcohols and amines.

Product	Size	Catalog #
MTBSTFA + 1% <i>t</i> -BDMCS	5 x 1 gram	270141
	10 x 1 gram	270144
	2 x 5 gram	270142
	25 gram	270143
MTBSTFA no <i>t</i> -BDMCS	5 x 1 gram	270241
	2 x 5 gram	270242
	25 gram	270243

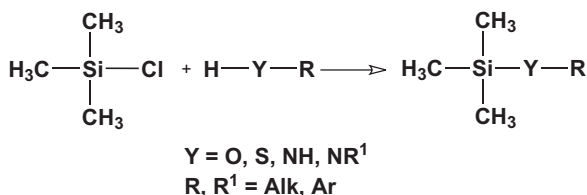


## TMCS

Trimethylchlorosilane

- Used as a catalyst to increase reactivity of other silylation reagents.

TMCS	25 gram	270601
	100 gram	270602

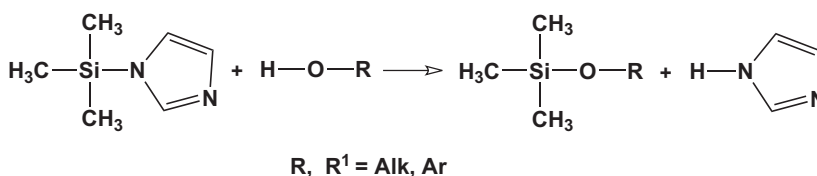


## TMSI

Trimethylsilylimidazole

- Potent, selective TMS donor that reacts with alcohols and phenols but not amines or amides.
- Derivatizes wet sugar samples, hindered hydroxyl groups in steroids, and amino acids in fluorinated acylation reagents.
- Used in the preparation of dual perfluoroacyl and TMS derivatives.

TMSI	10 x 1 gram	270401
	5 gram	270402
	25 gram	270403





# Silylation Formulations

Deriva-Sil	Product	Size	Catalog #
<ul style="list-style-type: none"> <li>• BSTFA:TMCS:TMSI:Pyridine (3:2:3:10) formulation.</li> <li>• Derivatizes sterically-hindered compounds.</li> <li>• Reacts with carbohydrates, hydroxy- and keto-steroids, fatty acids, and some amines and amides.</li> <li>• Derivatizations are complete in minutes.</li> </ul>	Deriva-Sil	10 x 1 ml	270151
	BSTFA:TMCS:TMSI:Pyridine (3:2:3:10)	25 ml	270152
<hr/>			
Deriva-Sil Concentrate	Deriva-Sil Concentrate	25 ml	270150
<ul style="list-style-type: none"> <li>• BSTFA:TMCS:TMSI (3:2:3) concentrate formulation.</li> <li>• Used for applications where pyridine is undesirable (i.e., 3-ketosteroids).</li> </ul>	BSTFA:TMCS:TMSI (3:2:3)		
	<hr/>		
Hydrox-Sil	Hydrox-Sil Reagent	25 ml	270457
<ul style="list-style-type: none"> <li>• HMDS:TMCS:Pyridine (2:1:10) formulation for one-step derivatizations.</li> <li>• Fast formation of the TMS derivatives of organic acids, unhindered alcohols and phenols, and some amines.</li> </ul>	HMDS:TMCS:Pyridine (2:1:10)		
	<hr/>		
Hydrox-Sil Concentrate	Hydrox-Sil Concentrate	25 ml	270458
<ul style="list-style-type: none"> <li>• HMDS:TMCS (2:1) concentrate formulation.</li> <li>• Suited for applications where pyridine in Hydrox-Sil is undesirable.</li> </ul>	HMDS:TMCS: (2:1)		
	<hr/>		

## Derivatization Grade Solvents

Derivatization Grade Solvents			
<ul style="list-style-type: none"> <li>• High purity reagents packaged under nitrogen.</li> <li>• Sealed with Teflon®-coated septa, allowing easy access to sample without exposure to moisture and oxygen.</li> </ul>	Acetonitrile	2 x 25 ml	270010
	Pyridine	2 x 25 ml	270013

# Acylation Reagents

## Fluorinated Anhydrides:

### HFBA/PFPA/TFAA

#### Heptafluorobutyric Anhydride/

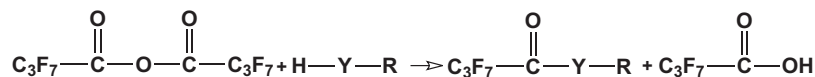
#### Pentafluoropropionic

#### Anhydride/Trifluoroacetic Anhydride

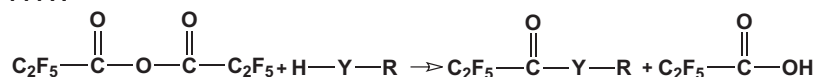
- Most commonly used for ECD.
- Reacts with alcohols, amines, and phenols.
- Bases such as triethylamine and trimethylamine can be added to promote reactivity.
- Frequently used for the confirmation of drugs of abuse.
- HFBA derivatives are the most sensitive to ECD.
- PFPA derivatives require the lowest analysis temperatures.
- TFAA is the most reactive and volatile of the anhydrides.

Product	Size	Catalog #
HFBA	10 x 1 gram	270851
	25 gram	270853
PFPA	10 x 1 gram	640110
	25 gram	640113
	100 gram	640114
TFAA	10 x 1 gram	270841
	25 gram	270843

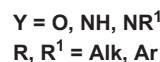
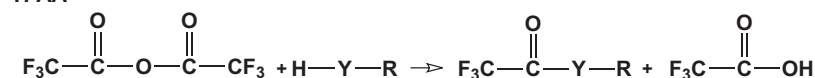
#### HFBA



#### PFPA



#### TFAA

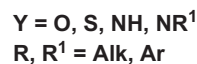
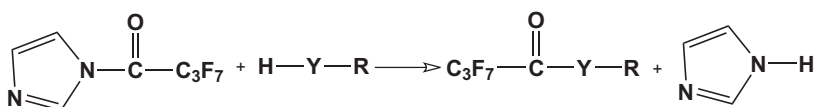


## HFBI

### Heptafluorobutyrylimidazole

- Readily forms derivatives with phenols, alcohols and amines suitable for ECD.
- Reactions are fast and mild.
- Imidazole is not acidic, so no decomposition or corrosion occurs on columns.

HFBI	5 x 1 gram	270611
	5 gram	270612

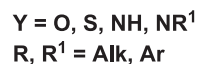
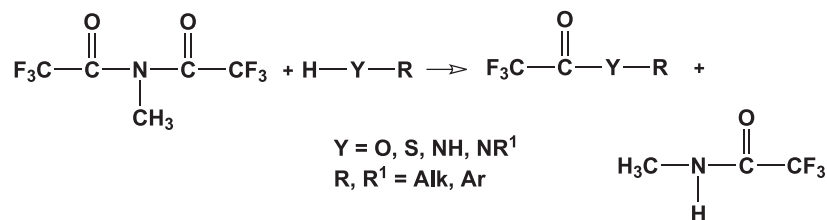


## MBTFA

### N-Methyl-N-bis(trifluoroacetamide)

- Reacts rapidly under mild conditions with primary and secondary amines.
- Reacts more slowly with alcohols, phenols, and thiols.
- Works well in the analysis of sugars.

MBTFA	10 x 1 gram	270092
	5 gram	270091
	25 gram	270095
	100 gram	270093

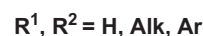
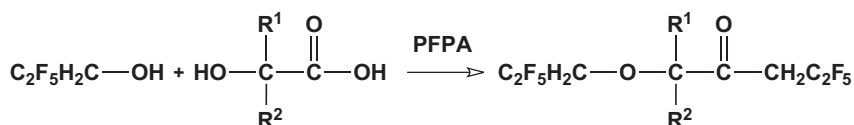


## PFPOH

### 2,2,3,3,3-Pentafluoropropanol

- Used in combination with PFPA to make derivatives of the most common functional groups, especially polyfunctional bio-organic compounds.
- Formed derivatives are highly suitable for ECD.

PFPOH	5 gram	270815
	25 gram	270816



# GC Chiral and Specialty Derivatization Reagents

TPC	Product	Size	Catalog #
<i>N</i> -Trifluoroacetyl-L-Prolyl Chloride	TPC	25 gram	440001
<ul style="list-style-type: none"> <li>Couples with amines to form diastereomers which can be separated on GC columns.</li> <li>Provides sample volatility.</li> <li>Used for confirmation of drugs of abuse testing.</li> </ul>	<p style="text-align: center;"><math>R^1, R^2, R^3 = H, \text{Alk}, \text{Ar}</math></p>		

MCF	Product	Size	Catalog #
(1 <i>R</i> , 2 <i>S</i> , 5 <i>R</i> )-(-)-Menthylchloroformate	MCF	25 gram	440003
<ul style="list-style-type: none"> <li>Resolves enantioenriched alcohols.</li> </ul>	<p style="text-align: center;"><math>R^1, R^2, R^3 = H, \text{Alk}, \text{Ar}</math></p>		

HFIP	Product	Size	Catalog #
1,1,1,3,3,3-Hexafluoro-2-Propanol	HFIP	25 gram 100 gram	270702 270704
<ul style="list-style-type: none"> <li>Esterification reagent for the determination of aromatic acids in tissue by GC and electron capture detection.</li> </ul>			
( <i>R</i> )-(-)-MTPA-Cl	( <i>R</i> )-(-)-MTPA-Cl	100 mg	270900
Mosher's acid chloride	Mosher's acid chloride	500 mg	270901
( <i>R</i> )-(-)- $\alpha$ -(trifluoromethyl)phenylacetyl chloride		1000 mg	270902

- (*R*)-(-)-MTPA-Cl is a ready to use reagent for the determination of enantiomeric purity of alcohols and amines.

# GC Chiral and Specialty Derivatization Reagents

## 3.0 N HCL in n-Butanol

- Most commonly used for rapid diagnosis of neonatal blood spots by Tandem Mass Spectrometry.

Product	Size	Catalog #
3.0N HCl in n-Butanol	4 x 25 ml	201007
	100 ml	201009
	500 ml	201010

3N HCl in n-Butanol is a derivatization reagent required for newborn screening for metabolic disorders. Neonatal screening, which has become a standard procedure in many countries, measures amino acids and acylcarnitines from a single drop of blood. Blood concentration of one or several of these compounds is either abnormally high or low in a variety of metabolic disorders in newborns. Derivatization with 3N HCl in n-Butanol ensures butylation of the carboxyl acid group of the analyte and formation of butyl ester, which forces ionization or makes charging of the analytes more efficient. Although direct analysis of extracted acycarnitine without derivatization is possible, according to different reports, butylesterification is superior with regard to sensitivity and specificity. Methods that include derivatization with 3N HCl in n-Butanol is the only validated procedure at this time.

Many factors contribute to the success of a newborn screening process. Any impurities in derivatization reagent can potentially interfere with the analysis. 3N HCl in n-Butanol from Regis Technologies is manufactured under cGMP protocols to assure highest quality and lot-to-lot consistency for this reagent. Each lot is tested by tandem mass spectrometry to ensure absence of contaminants which may interfere with analysis. Our Quality Assurance department reviews and approves all production documentation and test results. Regis takes necessary precautions that assure the quality of our 3N HCl in n-Butanol.





# GC Chiral and Specialty Derivatization Reagents

## Notes

For additional information on GC Derivatization Reagents, contact us by phone,

800.323.8144 ext. 662

847.967.6000 ext. 662

or e-mail,

[sales@registech.com](mailto:sales@registech.com)

Please note: Certain GC Derivatization products are hazardous chemicals and additional shipping and handling charges could apply.