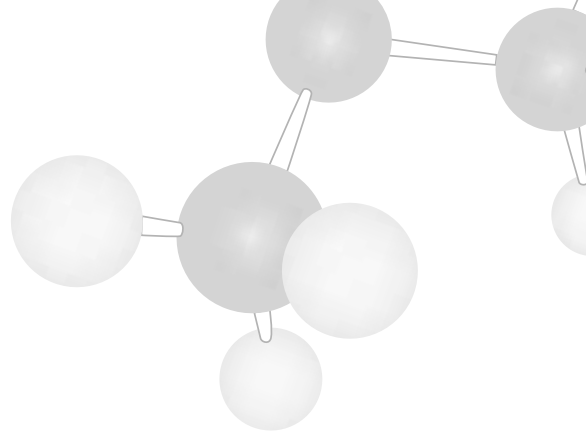


**GC
DERIVATIZATION
REAGENTS**



DERIVATIZATION



Derivatization

Derivatization is primarily performed to modify an analyte's functionality in order to enable chromatographic separations. For more than 30 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.



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*-butyldimethylsilyl (*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.

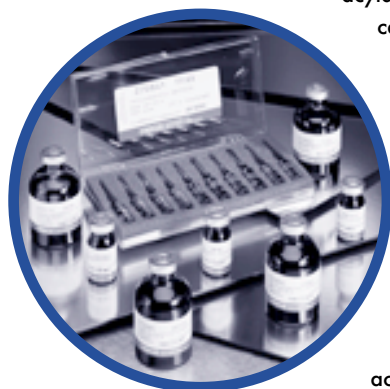
Both TMS and *t*-BDMS reagents are suitable for a wide variety of compounds, offer excellent thermal stability and can be used in a variety of GC conditions and applications.

Analysis by the popular combination of gas chromatography and mass spectrometry (GC/MS) often requires special sample derivatization. Particularly effective in these applications are MTBSTFA and deuterio-MTBSTFA-d.



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 electron-capturing groups, thus enhancing detectability during analysis.



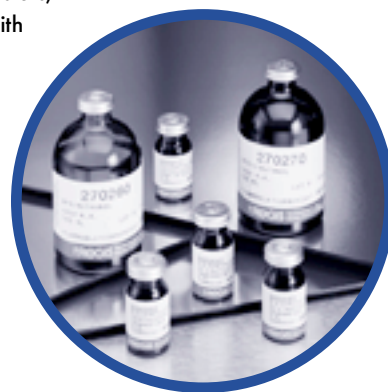
Generally, these reagents are available as acid anhydrides, acyl derivatives, or acyl halides. The acyl halides and acyl derivatives are highly reactive and are suitable for use where steric hindrance may be a factor. Acid anhydrides are supplied in a number of fluorinated configurations, which improve 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.

GAS CHROMATOGRAPHY (GC) DERIVATIZATION

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.



GUIDE TO GC DERIVATIZATION METHODS/FUNCTIONAL GROUPS

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

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.

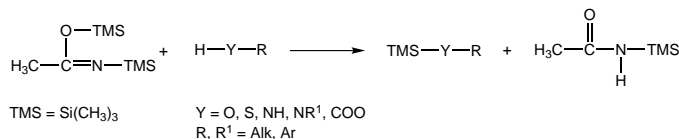
SILYLATION REAGENTS

BSA

N,O-Bis(trimethylsilyl)acetamide

- Forms highly stable TMS derivatives with most organic functional groups under mild reaction conditions.

Product	Size	Product #	U.S. Price
BSA	10 x 1 gm	270501	
	4 x 5 gm	270502	
	25 gm	270503	
	100 gm	270504	



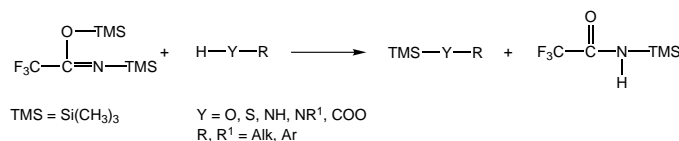
BSTFA-Regisil®

BSTFA +TMCS (1%, 10%)

N,O-Bis(trimethylsilyl)trifluoroacetamide

- 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.

Product	Size	Product #	U.S. Price
Regisil® RC-1 (BSTFA)	10 x 1 gm	270111	
	50 x 1 gm	270117	
	4 x 5 gm	270112	
	25 gm	270113	
Regisil® RC-2 (BSTFA) +1% TMCS	10 x 1 gm	270121	
	50 x 1 gm	270127	
	4 x 5 gm	270122	
	25 gm	270123	
Regisil® RC-3 (BSTFA) +10% TMCS	10 x 1 gm	270131	
	4 x 5 gm	270132	
	25 gm	270133	
	100 gm	270134	

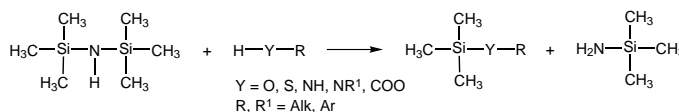


HMDS

Hexamethyldisilazane

- Weak TMS donor, used for silylation of carbohydrates.
- Used as mixture with pyridine and trifluoroacetic acid.

Product	Size	Product #	U.S. Price
HMDS	25 gm	270651	
	100 gm	270652	

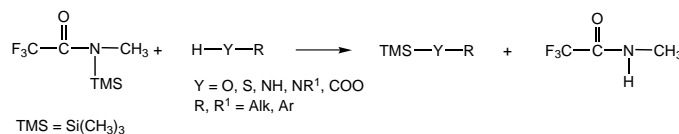


MSTFA

N-Methyltrimethylsilyltrifluoroacetamide

- Most volatile of the TMS-acetamides.
- Useful in the analysis of volatile trace materials.

Product	Size	Product #	U.S. Price
MSTFA	10 x 1 gm	270590	
	10 gm	270589	
	25 gm	270593	
	100 gm	270594	



SILYLATION REAGENTS

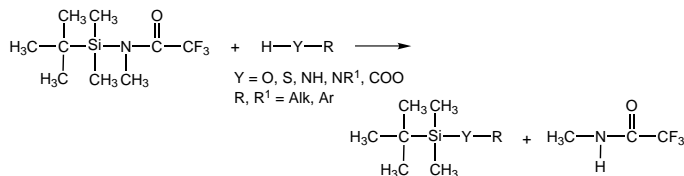
MTBSTFA

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	Product #	U.S. Price
MTBSTFA	5 x 1 gm	270141	
(+ 1% <i>t</i> -BDMCS)	10 x 1 gm	270144	
	2 x 5 gm	270142	
	25 gm	270143	
MTBSTFA	5 x 1 gm	270241	
(w/ <i>t</i> -BDMCS)	2 x 5 gm	270242	
	25 gm	270243	

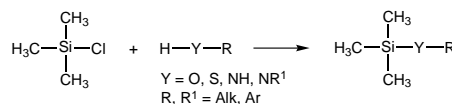


TMCS

Trimethylchlorosilane

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

Product	Size	Product #	U.S. Price
TMCS	25 gm	270601	
	100 gm	270602	

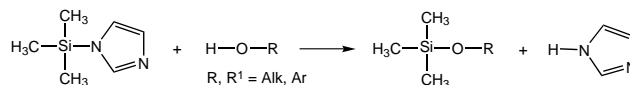


TMSI

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.

Product	Size	Product #	U.S. Price
TMSI	10 x 1 gm	270401	
	5 gm	270402	
	25 gm	270403	



SILYLATION FORMULATIONS

Deriva-Sil

- BSTFA:TMCS:TMSI:Pyridine (3:2:3:10) formulation.
- Derivatizes sterically-hindered compounds.
- Reacts with carbohydrates, hydroxy- and keto-steroids, fatty acids, and some amines and amides.
- Derivatizations are complete in minutes.

Product	Size	Product #	U.S. Price
Deriva-Sil –	10 x 1 mL	270151	
BSTFA:TMCS:	25 mL	270152	
TMSI:Pyridine			
(3:2:3:10)			

Deriva-Sil Concentrate

- BSTFA:TMCS:TMSI (3:2:3) concentrate formulation.
- Used for applications where pyridine is undesirable (*i.e.*, 3-ketosteroids).

Product	Size	Product #	U.S. Price
Deriva-Sil Concentrate –	25 mL	270150	
BSTFA:TMCS:TMSI			
(3:2:3)			

Product information and applications are available online at:
www.registech.com/gc/.

SILYLATION FORMULATIONS

Hydrox-Sil

- HMDS:TMCS:Pyridine (2:1:10) formulation for one-step derivatizations.
- Fast formation of the TMS derivatives of organic acids, unhindered alcohols and phenols, and some amines.

Product	Size	Product #	U.S. Price
Hydrox-Sil – Reagent	10 x 1 mL	270455	
HMDS:TMCS:Pyridine (2:1:10)	25 mL	270457	

Hydrox-Sil AQ

- TMSI:Pyridine (21%w/v) formulation.
- Forms TMS derivatives of hydroxyl and polyhydroxyl compounds in the presence of water.

Product	Size	Product #	U.S. Price
Hydrox-Sil AQ –	10 x 1 mL	270451	
TMSI:Pyridine (21% w/v)	25 mL	270453	

Hydrox-Sil Concentrate

- HMDS:TMCS (2:1) concentrate formulation.
- Suited for applications where pyridine in Hydrox-Sil is undesirable.

Product	Size	Product #	U.S. Price
Hydrox-Sil Concentrate –	25 mL	270458	
HMDS:TMCS (2:1)			

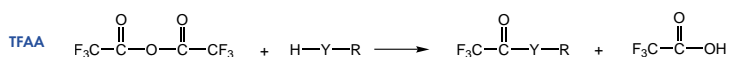
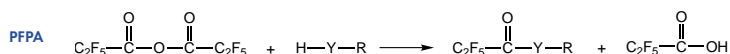
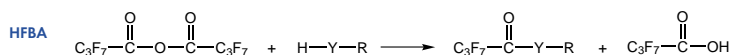
ACYLATION REAGENTS

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	Product #	U.S. Price
HFBA	10 x 1 gm	270851	
	25 gm	270853	
PFPA	10 x 1 gm	640110	
	25 gm	640113	
	100 gm	640114	
TFAA	10 x 1 gm	270841	
	25 gm	270843	



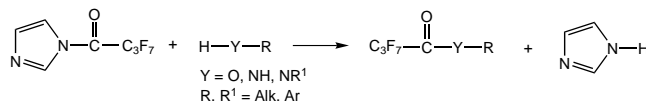
Y = O, NH, NR¹
R, R¹ = Alk, Ar

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.

Product	Size	Product #	U.S. Price
HFBI	5 x 1 gm	270611	
	5 gm	270612	



GC CHIRAL AND SPECIALTY DERIVATIZATION REAGENTS

HFIP

1,1,1,3,3,3-Hexafluoro-2-Propanol

- Esterification reagent for the determination of aromatic acids in tissue by GC and electron capture detection.

Product	Size	Product #	U.S. Price
HFIP	10 gm	270701	
	25 gm	270702	
	4 x 25 gm	270703	
	100 gm	270704	

3.0 N HCL in n-Butanol

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

Product	Size	Product #	U.S. Price
3.0 N HCL in n-Butanol	4 x 25 mL	201007	
	100 mL	201009	
	500 mL	201010	

Col-Treet

- Silylating mixture designed for conditioning gas chromatography columns.
- Eliminates peak tailing, peak broadening and residues which may reduce column efficiency.
- Not to be used with any hydrogen bonding stationary phases such as CARBOWAX® or phases with active hydrogen sites.

Product	Size	Product #	U.S. Price
Col-Treet	10 x 1 mL	970130	
	4 x 5 mL	970131	

Glas-Treet

- Used to deactivate active sites found in glassware, chromatography columns, and glass wool.
- Does not coat glass; renders it inactive chemically.

Product	Size	Product #	U.S. Price
Glas-Treet	100 mL	970068	

CARBOWAX is a registered trademark of Union Carbide Corp.

For additional information on GC Derivatization Reagents, check our Web site at www.registech.com/gc/ or contact Regis directly at:

(800) 323-8144 ext. 649

(847) 967-6000 ext. 649

e-mail us at: sales@registech.com.

