



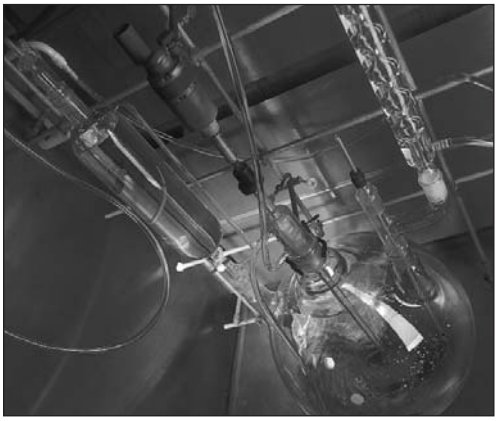
GC Derivatization Reagents

Derivatization

Derivatization is primarily performed to modify an analyte's function ality 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.



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 (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.

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

GC Derivatization Method

Functional Group	Silylation	Acylation	Alkylation
Active Hydrogens	BSTFA, BSTFA/TMCS, Deriva-Sil, Hydrox-Sil, TBH MSTFA, MTBSTFA, TMSI	PFPOH/PFPA	DMF Dialkylacetals,
Carboxylic Acids	BSTFA, Hydrox-Sil Conc., MTBSTFA, TMSI	PFPOH/PFPA	BF_3/Methanol, BF_3/n-Butanol, DMF Dialkylacetals, TBH
Alcohols and Phenols: unhindered and moderately hindered	BSA, BSTFA/TMCS, HMDS, MTBSTFA/*t*-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, PFBCl	DMF Dialkylacetals, PFB-Br/TBA-H-SO_4, TBH
Amines: primary and secondary	BSTFA, MTBSTFA/*t*-BDMCS	Fluorinated anhydrides (HFBA, PFPA, TFAA), HFBI, MBTFA, PFBCl, TPC*	DMF Dialkylacetals, TBH
Amides	BSA, BSTFA, BSTFA/TMCS, Deriva-Sil Conc.	HFBI	DMF Dialkylacetals,
Amino Acids	BSTFA, TMSI	HFBI (+ Silylation)	DMF Dialkylacetals, TBH 3N HCl in n-Butanol
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.

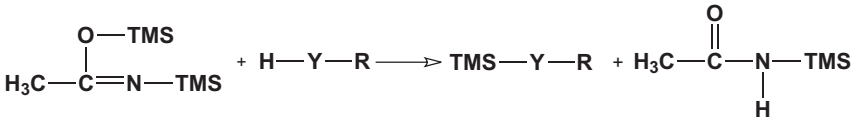
Silyation Reagents

BSA

N,O-Bis(trimethylsilyl)acetamide

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

Product	Size	Catalog #
BSA	10 x 1 gram	270501
	4 x 5 gram	270502
	25 gram	270503
	100 gram	270504



TMS= $Si(CH_3)_3$

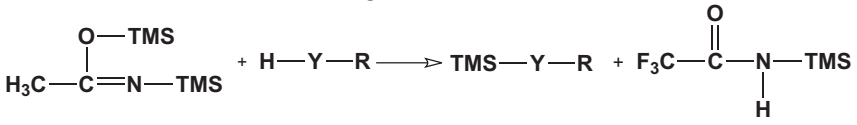
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

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	Catalog #
Regisil® RC-1 BSTFA	10 x 1 gram	270111
	4 x 5 gram	270112
	25 gram	270113
	100 gram	270114
	1000 gram	270116
Regisil® RC-2 BSTFA +1% TMCS	10 x 1 gram	270121
	4 x 5 gram	270122
	25 gram	270123
	100 gram	270124
	1000 gram	270126
Regisil® RC-3 BSTFA +10% TMCS	10 x 1 gram	270131
	4 x 5 gram	270132
	25 gram	270133
	100 gram	270134
	1000 gram	270135



TMS= $Si(CH_3)_3$

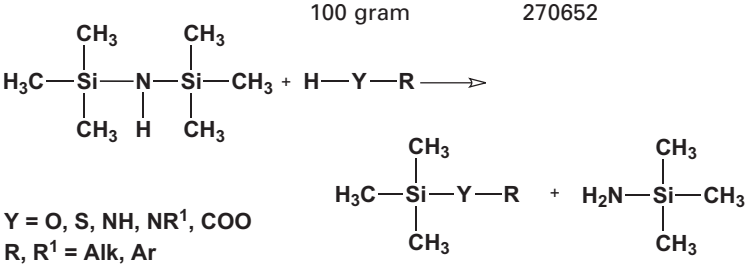
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

HMDS

Hexamethyldisilazane

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

Product	Size	Catalog #
HMDS	25 gram	270651
	100 gram	270652



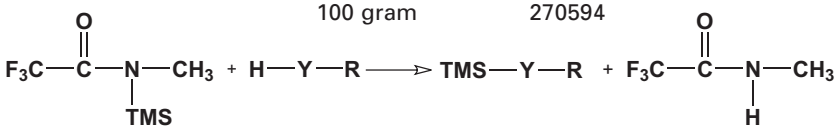
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

MSTFA

N-Methyltrimethylsilyltrifluoroacetamide

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

Product	Size	Catalog #
MSTFA	10 x 1 gram	270590
	10 gram	270589
	25 gram	270593
	100 gram	270594



TMS= $Si(CH_3)_3$

Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

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Silyation Reagents

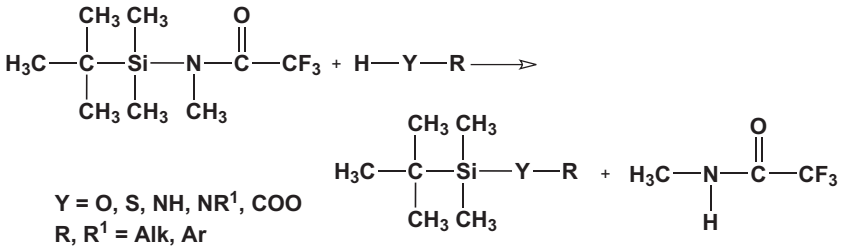
MTBSTFA

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% t-BDMCS	5 x 1 gram	270141
	10 x 1 gram	270144
	2 x 5 gram	270142
	25 gram	270143
MTBSTFA no t-BDMCS	5 x 1 gram	270241
	2 x 5 gram	270242
	25 gram	270243



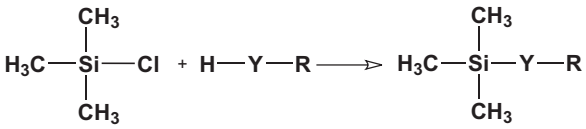
Y = O, S, NH, NR^1, COO
R, R^1 = Alk, Ar

TMCS

Trimethylchlorosilane

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

Product	Size	Catalog #
TMCS	25 gram	270601
	100 gram	270602



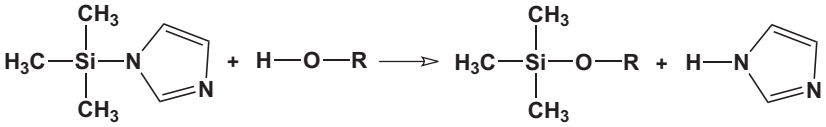
Y = O, S, NH, NR^1
R, R^1 = Alk, Ar

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



R, R^1 = Alk, Ar

Silyation Formulations

Deriva-Sil	Product	Size	Catalog #
• 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.	Deriva-Sil BSTFA:TMCS: TMSI:Pyridine (3:2:3:10)	10 x 1 ml 25 ml	270151 270152
Deriva-Sil Concentrate			
• BSTFA:TMCS:TMSI (3:2:3) concentrate formulation. • Used for applications where pyridine is undesirable (i.e., 3-ketosteroids).	Deriva-Sil Concentrate BSTFA:TMCS:TMSI (3:2:3)	25 ml	270150
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.	Hydrox-Sil Reagent HMDS:TMCS:Pyridine (2:1:10)	25 ml	270457
Hydrox-Sil Concentrate			
• HMDS:TMCS (2:1) concentrate formulation. • Suited for applications where pyridine in Hydrox-Sil is undesirable.	Hydrox-Sil Concentrate HMDS:TMCS: (2:1)	25 ml	270458

Derivatization Grade Solvents

Derivatization Grade Solvents			
• High purity reagents packaged under nitrogen. • Sealed with Teflon®-coated septa, allowing easy access to sample without exposure to moisture and oxygen.	Acetonitrile	2 x 25 ml	270010
	Pyridine	2 x 25 ml	270013

Acylation Reagents

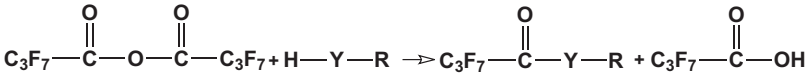
Fluorinated Anhydrides: HFBA/PFPA/TFAA

Heptafluorobutyric Anhydride/ Pentafluoropropionic Anhydride/Trifluoroacetic Anhydride

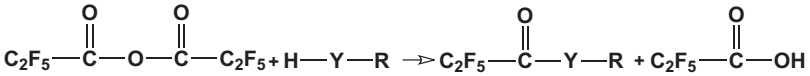
- Most commonly used for ECD.
- Reacts with alcohols, amines, and phenols.
- Bases such as triethylamine and trimethylamine can be added to promote reactivity.
- Frequently used for the confirmation of drugs of abuse.
- HFBA derivatives are the most sensitive to ECD.
- PFPA derivatives require the lowest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

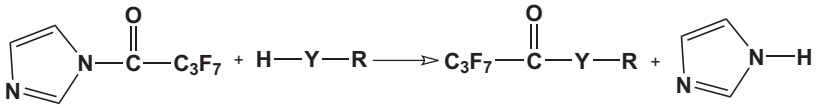
Y = O, NH, NR^1
R, R^1 = Alk, Ar

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	Catalog #
HFBI	5 x 1 gram	270611
	5 gram	270612



Y = O, S, NH, NR^1
R, R^1 = Alk, Ar

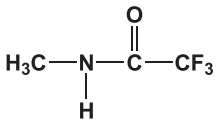
MBTFA

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.

Product	Size	Catalog #
MBTFA	10 x1 gram	270092
	5 gram	270091
	25 gram	270095
	100 gram	270093

Y = O, S, NH, NR^1
R, R^1 = Alk, Ar

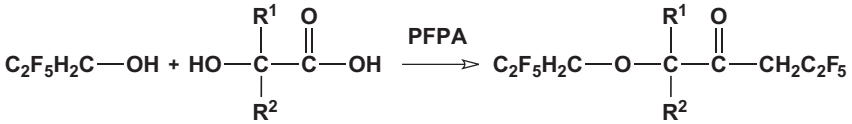


PFPOH

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.

Product	Size	Catalog #
PFPOH	5 gram	270815
	25 gram	270816



R^1, R^2 = H, Alk, Ar

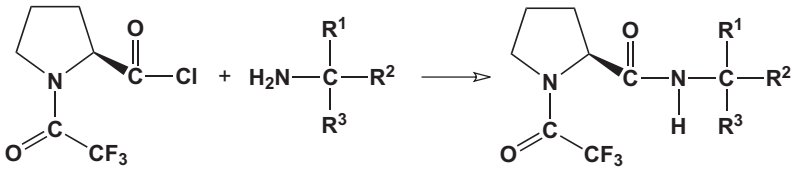
GC Chiral and Specialty Derivatization Reagents

TPC

N-Trifluoroacetyl-L-Prolyl Chloride

- Couples with amines to form diastereomers which can be separated on GC columns.
- Provides sample volatility.
- Used for confirmation of drugs of abuse testing.

Product	Size	Catalog #
TPC	25 gram	440001



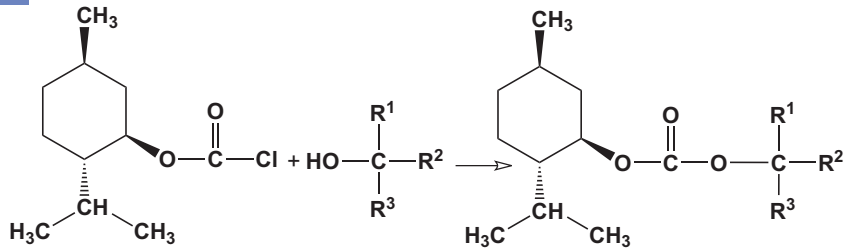
R^1, R^2, R^3 = H, Alk, Ar

MCF

(1R, 2S, 5R)-(-)-Menthylchloroformate

- Resolves enantioenriched alcohols.

Product	Size	Catalog #
MCF	25 gram	440003



R^1, R^2, R^3 = H, Alk, Ar

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	Catalog #
HFIP	25 gram	270702
	100 gram	270704

(R)-(-)-MTPA-Cl

Mosher's acid chloride
(R)-(-)-α-(trifluoromethyl) phenylacetyl chloride

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

Product	Size	Catalog #
(R)-(-)-MTPA-Cl Mosher's acid chloride	100 mg	270900
	500 mg	270901
	1000 mg	270902

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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.