

Analytical Derivatization for Gas Chromatography

Derivatization

Definition

- The chemical modification of an existing compound to produce a new compound having properties that are suitable for a specific analytical procedure.

Analytical Derivatization

What are the Reasons for Derivatization?

- Impart Volatility
- Decrease Adsorption
- Improve Resolution
- Increase Stability
- Improve Detectability
- Assist in Structure Determination

Requirements

The Ideal Derivatization Procedure Will...

- Accomplish the desired modification.
- Proceed quantitatively, or at least reproducibly.
- Produce products which are readily distinguishable and separable from the starting materials.
- Proceed rapidly with simple and straightforward laboratory techniques.
- Be relatively selective while being applicable to a number of similar compounds.
- Involve reagents and reactions which present no unusual hazards.

A General Reaction Scheme

The Ideal Derivatization Procedure Will...

The most commonly used derivatization procedures involve the substitution of active hydrogens on the compound to be derivatized with a variety of functional groups. These functional groups impart the desired characteristics to the compound, while eliminating the adverse effects of the polar active hydrogens.



Where atom "A" = Oxygen, Sulfur, Nitrogen or similar atoms

Where atom "D" = Functional group on the derivatization reagent

Derivatization Techniques

Reaction Types

- Silylation
- Acylation
- Alkylation
- Specialized

Silylation

Definition

- The introduction of the silyl group into a molecule, usually by substitution of active hydrogen; occasionally by replacement of the metal component of a salt.

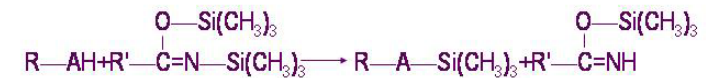
The most frequently used derivatives for gas chromatography analysis are:

Trimethylsilyl — $\text{Si}(\text{CH}_3)_3$

t-Butyldimethylsilyl — $\text{Si}(\text{CH}_3)_2\text{-C}(\text{CH}_3)_3$

Silylation

General Reaction Scheme



Where A = O, S, N, or similar atoms.

Examples: Trimethylsilyl (TMS) = $\text{Si}(\text{CH}_3)_3$



Silylation

Advantages

- Wide range of applications
- Variety of reagents available
- Easily prepared
- Excellent thermal stability
- Excellent chromatographic characteristics

Disadvantages

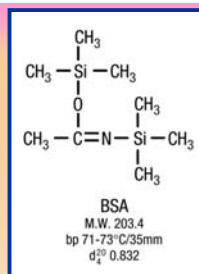
- Silylation reagents are moisture-sensitive
- TMS and TBDMCS derivatives are easily hydrolyzed
- Derivatives cannot be made in aqueous solutions
- Must use aprotic organic solvents
- Silylating reagents and silyl derivatives react with many column materials
- Silicone residues build up in GC detectors

Commonly Used Silylation Reagents

- | | | |
|---------|---|---|
| BSA | } | Frequently used with TMCS Catalyst |
| BSTFA | | |
| MSTFA | | |
| HMDS | } | Usually used together for carbohydrates |
| TMCS | | |
| TMSI | } | Good for volatile carboxylic acids |
| TMSDEA | | |
| MTBSTFA | } | Frequently used with TBDMCS Catalyst |

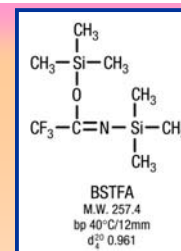
BSA

- **Strong silyl donor**
 - Similar to BSTFA and MSTFA
- **Reacts with all active hydrogen compounds**
 - Alcohols, phenols, carboxylic acids, amines, amides, thiols
- **Usually requires anhydrous condition**
- **TMCS 1% - 10% frequently used as catalyst**
- **Sometimes reacts quantitatively under mild conditions**
- **Reaction products often interfere with volatile derivatives**
- **Silicon fouling of detectors is common**

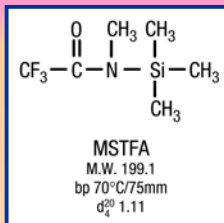


BSTFA

- **Strong silyl donor**
 - Similar to BSA and MSTFA
- **Frequently used with TMCS 1 - 10%**
- **Alone and with TMCS, most commonly used derivatizing agent**
- **Reacts with all active hydrogen compounds**
 - Alcohol, phenols, carboxylic acids, amines, amides, thiols
- **Usually requires anhydrous condition**
- **Often reacts quantitatively under mild conditions**
- **Reaction products more volatile than those from BSA**
- **Much less detector fouling than with BSA**



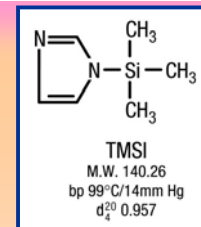
MSTFA



PIERCE

- Strong silyl donor
 - Similar to BSA and BSTFA – always monovalent
- Frequently used with TMCS 1 - 10%
- Reacts with all active hydrogen compounds
 - Alcohols, phenols, carboxylic acids, amines, amides, thiols
- Most volatile reagent and reaction product
 - Used for derivatizing small volatile molecules
- Better than BSA in avoiding detector fouling
- Usually requires anhydrous conditions

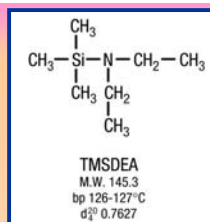
TMSI



PIERCE

- Strongest silyl donor for hydroxyl groups
- Reacts quickly and smoothly with hydroxyl and carboxyl
- Does not react with amines or amides
- Can derivatize hydroxyl in the presence of amines
- Permits selection or successive derivatization of hydroxyls and amines
- Preferred reagent for sugar
- Can tolerate small amounts of water as in syrups
- Can derivatize even highly hindered hydroxyls

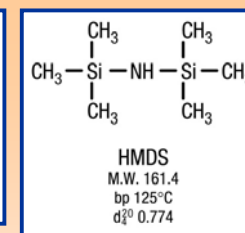
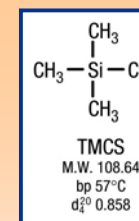
TMSDEA



PIERCE

- Strongly basic silylating agent
- Very volatile reagent
- Excellent for derivatizing low molecular weight carboxylic acids
- Reaction can be driven to completion by removal of diethylamine (bp 55°C)
- Good for preparation of TMS standards
- Relatively weak silyl donor

TMCS and HMDS

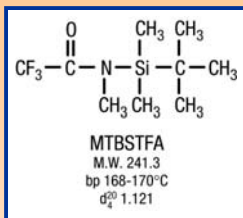


PIERCE

- Weak silyl donors
- Among the oldest (first) silylating reagents
- Usually used in combination with each other
- Excellent for derivatization of sugars and simple carbohydrates
- Usually combined with pyridine and other solvents
- TMCS can form derivatives of sodium salts of acids and phenols

MTBSTFA

- Produces t-butyldimethylsilyl (TBDMS) derivatives
- Strong silyl donor
 - Slightly less than BSA, BSTFA, MSTFA
- Reacts with all active hydrogen compounds
 - Alcohols, phenols, carboxylic acids, amines, thiols
- Derivative much more hydrolytically stable than TMS
- Produces characteristic fragmentation patterns on GC/MS
- Bulky group, reaction may be difficult due to steric hindrance
- Frequently used with t-butyldimethylchlorosilane (TBDMCS) as a catalyst



Ready-to-Use Tri-Sil[®] Reagents

Reagent	Description	Formulation	Applications
Tri-Sil [®] 48999	A reagent-catalyst-solvent mixture for one-step derivatization	HMDS:TMCS: Pyridine (3:1:9)	Carbohydrates, phenols, steroids, organic acids, alcohols, and some amines. Not recommended for 3-keto steroids.
Tri-Sil [®] Concentrate 49005	A concentrated reagent-catalyst system	HMDS:TMCS (3:1)	Has same applications as Tri-Sil [®] , but offers greater latitude in applications (e.g. pyridine for sugars, DMF for 3-keto steroids, and DMSO for tertiary alcohols).
Tri-Sil [®] BSA Formula "P" 49011	A reagent-solvent system where BSA is the active silylating agent. A one-step derivatizing system.	BSA:Pyridine Hydroxy and (2.5 mEq/ml)	polyhydroxy compounds, amines, acids, amides, phenols, amino acids, carboxylic acids and steroids. Not recommended for carbohydrates.
Tri-Sil [®] BSA Formula "D" 49010	A reagent-solvent system where BSA is the active silylating agent. A one-step derivatizing system.	BSA:DMF (2.5 mEq/ml)	Same applications as Tri-Sil [®] BSA Formula "P" above, but where DMF is recommended as a solvent. A preferred formulation for phenols, particularly highly hindered phenols, where DMF is required for a smooth complete reaction.

Ready-to-Use Tri-Sil[®] Reagents (continued)

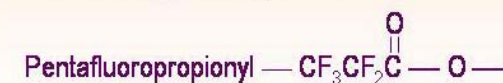
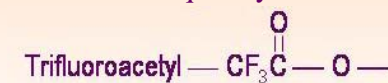
Reagent	Description	Formulation	Applications
Tri-Sil [®] "BT" 49015	A mixture of BSA and trimethylchlorosilane (TMCS). Use with: acetonitrile, DMF, pyridine, or other solvents if desired. If DMF is used, discard mixture after 12 hours.	BSA:TMCS: (5:1)	Unhindered and moderately hindered hydroxy groups
Tri-Sil [®] "TBT" 49016	A powerful silylating mixture composed of trimethylsilylimidazole (TMSI), N,O-bis(trimethylsilyl)acetamide (BSA) and trimethylchlorosilane (TMCS). Use with acetonitrile, DMF, pyridine or other solvents if desired. If DMF is used, discard mixture after 12 hours.	TMSI:BSA:TMCS (3:3:2)	All hydroxyl groups
Tri-Sil [®] "Z" 49230	A reagent-solvent system where trimethylsilylimidazole is the active silylating agent. A one-step derivatizing system.	TMSI:Pyridine (1:4)	Hydroxy and polyhydroxy compounds, will not derivatize amino groups. Reagent of choice for carbohydrates — wet or dry. Hindered and unhindered steroids.

Acylation

Definition

- The introduction of an acyl moiety into a molecule via substitution of an active hydrogen

The most frequently used derivatives for gas chromatography:



Acylation

General Reaction Scheme



where $\text{R}'-\overset{\text{O}}{\parallel}{\text{C}}-\text{X}$ is the acylating agent

Acylation

Advantages

- Derivatives are hydrolytically stable
- Perfluoro derivatives increase volatility
- Increased sensitivity by added molecular weight
- Increased detectability by ECD by added halogen atoms
- Reacts with alcohols, thiols and amines
- Can be used to activate carboxylic acids for esterification

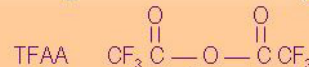
Disadvantages

- Derivatives are frequently difficult to prepare
- Reaction products often must be removed before analysis
- Reaction must be done in non-aqueous system
- Reagents are moisture-sensitive
- Reagents are hazardous and odorous

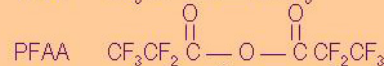
Commonly Used Acylation Reagents

Perfluoroacyl Anhydrides

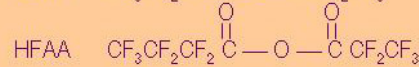
Trifluoroacetic Acid Anhydride



Pentafluoropropionic Acid Anhydride

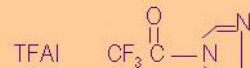


Heptafluorobutyric Acid Anhydride

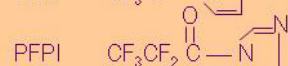


Perfluoroacyl Imidazoles

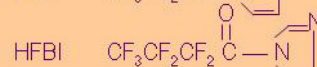
Trifluoroacetylimidazole



Pentafluoropropionylimidazole

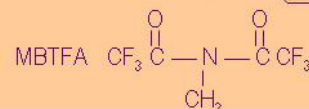


Heptafluorobutyrylimidazole



MBTFA

N-methyl-bis-(trifluoroacetamide)



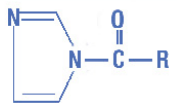
Perfluoro Acid Anhydrides

- Produce perfluoroacyl derivatives of alcohols, thiols and amines
- Derivatives are relatively stable to hydrolysis
- Derivatives are useful for ECD, FID and TCD detection
- Usually used with basic solvent
- Produce characteristic MS fragmentation analysis
- Are widely used for drug analysis
- Produce acid byproducts which must be removed before GC analysis

R	Name	MW	bp	d ₄ ²⁵
CF ₃	TFAA	210.0	39-40°C	1.490
C ₂ F ₅	PFAA	310.0	74-75°C	1.571
C ₃ F ₇	HFAA	410.0	106-107°C	1.665

Perfluoroacylimidazoles

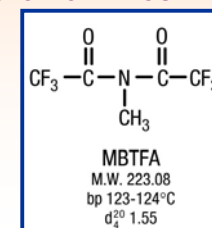
- Produce perfluoro derivatives of alcohols, amines and thiols
- Quantitatively acylate indol alkylamines
- Derivatives are relatively stable to hydrolysis
- Derivatize both primary and secondary amines
- Produce no acidic byproducts
- Reagents are very reactive with water
- Substance to be derivatized must be dry
- Cannot use in protonated solvents



R	Name	MW	bp	d ₄ ²⁵
CF ₃	TFAI	164.08	38-40°C/14mm	1.490
C ₂ F ₅	PFAI	214.10	38-40°C/14mm	1.428
C ₃ F ₇	HFBI	264.10	57-58°C/10mm	1.562

MBTFA N-Methyl-bis(Trifluoroacetamide)

- Forms trifluoroacetyl derivatives of alcohols, amines and thiols
- Reacts with both primary and secondary amines
- Reactions with amines generally complete in 30 minutes at room temperature
- Reacts more slowly with alcohols than amines
- Byproduct is stable and volatile
- Excellent for mono-, di- and trisaccharides



Alkylation

Definition

- The introduction of an alkyl moiety into a molecule via substitution of an active hydrogen

The most frequently used derivatives for gas chromatography analysis are:

Methyl — CH₃

Perfluorobenzyl — 

Alkylation

General Reaction Scheme



Where R' — X = the alkylating reagent

Alkylation

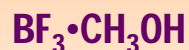
- **Advantages**
 - Wide range of reagents available
 - Wide range of derivatives can be produced
 - Reaction condition can vary from strongly acidic to strongly basic
 - Some reactions can be done in aqueous systems
 - Derivatives are generally stable
- **Disadvantages**
 - Limited to amines and acidic hydroxyls
 - Conditions frequently severe
 - Reagents often toxic
 - Optimization for particular compounds usually necessary

Commonly Used Alkylation Reagents

- **BF₃ • Methanol**
- **Methyl 8[®] Reagent**
- **MethElute[™] Reagent**
- **Diazomethane**
- **Pentafluorobenzyl Bromide**

Alkylating Reagents

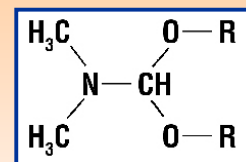
Boron Trifluoride in Methanol



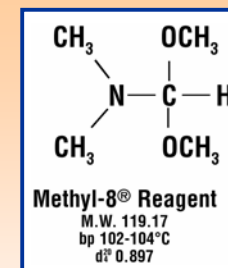
Used primarily for esterification procedures with fatty acids; however, phenolic hydroxyls may be derivatized

Alkylating Reagents

Dimethylformamide Dialkylacetals



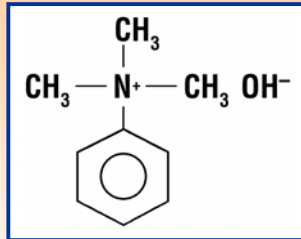
R = Methyl
Ethyl
Propyl
n-Butyl



Used for carboxylic acid esterification. Analytical applications have been expanded to include alcohols, phenols, steroid carbonyls, amino acids, primary and secondary amines, and thiols.

Alkylating Reagents

Trimethylanilinium Hydroxide TMPAH

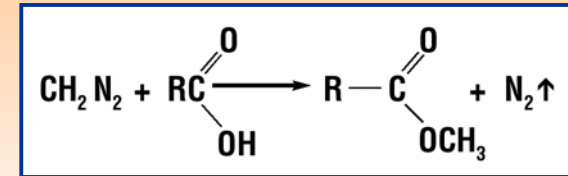


MethElute™ Reagent – 0.2M TMPAH in Methanol.

Used for on-column methylation of amines, hydroxyls and carboxyls.

Alkylation Reagents

Diazomethane

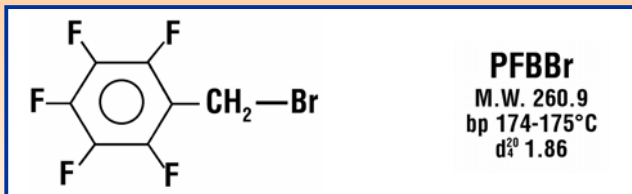


Most versatile reagent for preparation of methyl esters; fast and quantitative with no organic byproducts.

Diazomethane and its precursors are toxic and dangerous.

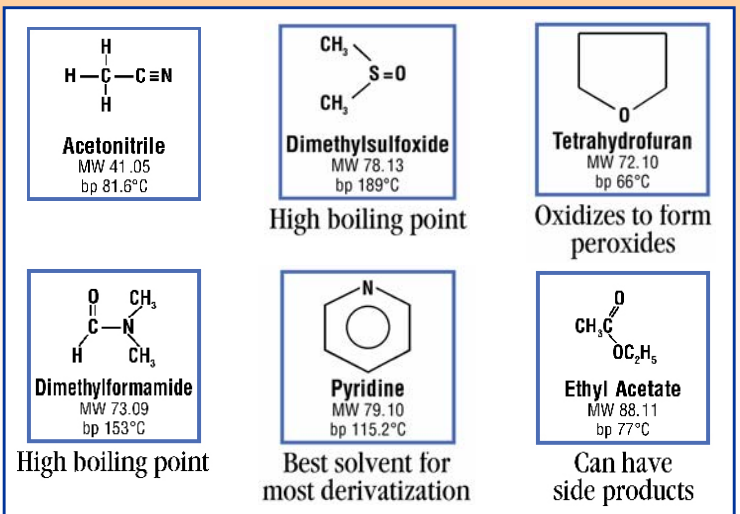
Alkylating Reagents

Pentafluorobenzyl Bromide



Used for the preparation of pentafluorobenzyl derivatives of carboxylic acids, phenols, sulfonamides and some mercaptans. Can be used for determination of trace amounts of carboxylic acids, phenols, and mercaptans in water. Most useful for ECD detection due to the introduction of five fluorine atoms.

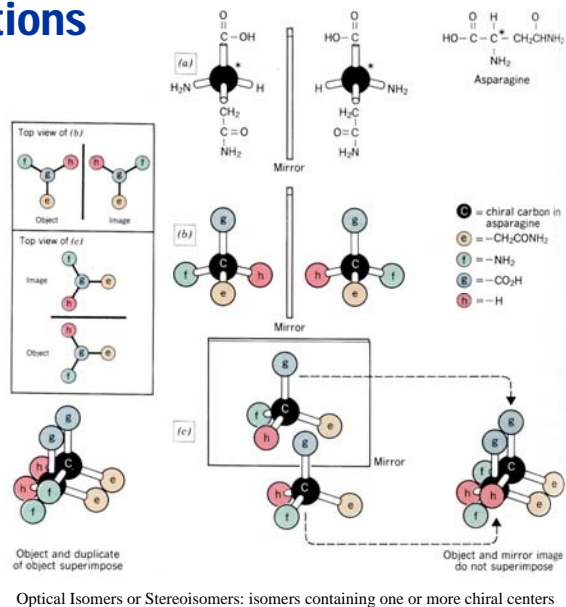
Solvents for Derivatization



Chiral Separations

Chiral Chemistry

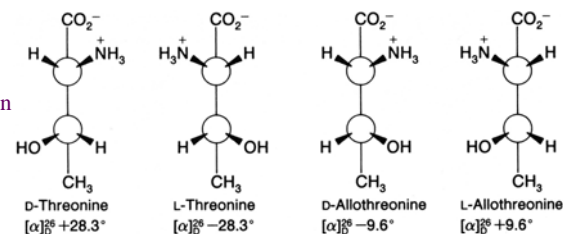
- Isomers: molecules that have the same molecular formula but a different arrangement of atoms.
- Chiral centers or asymmetric carbons: carbon atoms having four different groups or atoms attached.



Types of Optical Isomers

Enantiomers

- Isomers that are mirror images of each other but can not be superimposed

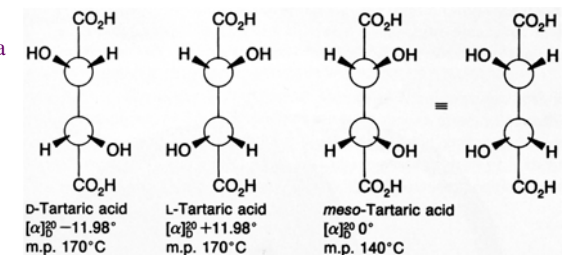


Diastereomers

- Stereoisomers that are not mirror images of each other

Meso Compounds

- Sets of stereoisomers with a plane of symmetry making them not optically active



If n is the number of chiral centers, then 2^n is the number of stereoisomers

Separation of Chiral Compounds

Diastereomers

- Diastereomers may have different chemical and physical properties and can usually be separated by classical methods

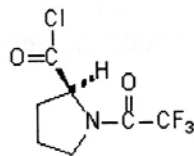
Enantiomers

- Enantiomers have identical chemical and physical properties except for their ability to rotate the plane of polarized light. Special techniques must be used for separation and identification

Chromatographic separation of enantiomers

Separation on chiral column

Pre-column derivatization with chiral derivatizing reagents, then separation on chromatographic columns



S-(-)-N-(trifluoroacetyl)-propyl chloride (S-(-)-TFAPC)

(-)- α -methoxy- α -trifluoromethylphenylacetic acid (MTPA), (S)-(-)-N-(trifluoroacetyl)propyl chloride (L-TPC); 2,3,4,6-tetra-O-acetyl- β -D-glucopyranosyl isothiocyanate, R-(+)- α -phenylethyl isocyanate, 2,3,4-triacetyl- α -D-arabinopyranosyl isothiocyanate

Chiral Drugs

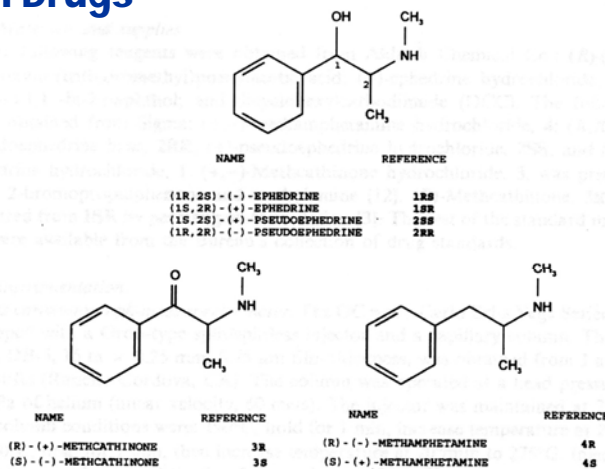


Fig. 1. Structures and stereochemical notation for ephedrine, pseudoephedrine, methcathinone and methamphetamine.

To Derivatize or not to Derivatize: That is the Question

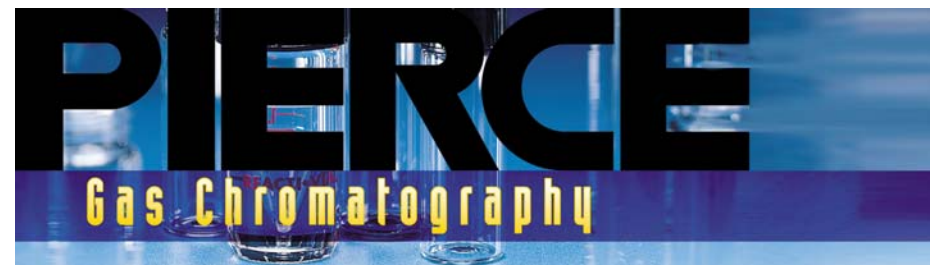
I. Review of Gas Chromatography

- A. Basic Chromatography
- B. Gas Chromatography
- C. Introduction to GC/MS

II. Analytical derivatization for Gas Chromatography

- A. What is Analytical Derivatization and Why we do it
- B. General requirements and Chemical Reactions
- C. Derivatization Techniques
 - 1. Silylation
 - 2. Acylation
 - 3. Alkylation
 - 4. Specialized, including Chiral

III. Summary



PIERCE
Gas Chromatography

Quality and Experience ...
They are what separate Pierce GC products from the competition

When Dr. Alan Pierce founded Pierce Chemical Company in 1950, Pierce was the first commercial producer of ninhydrin, a chemical used for amino acid analysis. He soon expanded the product line to include fluorine compounds and organic silicone compounds used for chromatography.

Dr. Pierce, who authored the famous textbook *Silylation of Organic Compounds*, was recognized throughout the industry not only as an expert in GC, but also as a teacher and a scientist who cared about his customers. Although Pierce product lines have grown and diversified during the past 52 years, the scientists of Pierce Biotechnology (formerly Pierce Chemical Company) are proud to continue Dr. Pierce's tradition of providing the GC reagents and solvents – and other products – that scientists around the world trust for performance and reliability.

Silylation, Alkylation and Acylation Reagents • Purified Silylation Grade Solvents
GC Derivatization Systems • Labware

Pierce Biotechnology, Inc. • 3747 N. Meridian Rd. • Rockford, IL 61105 U.S.A.
Tel: 815-968-0747 or 800-874-3723 • Fax: 815-968-7316 • Internet: www.piercenet.com
Technical Assistance E-mail: TA@piercenet.com • Customer Assistance E-mail: CS@piercenet.com

