Derivatization reagents

Key features

- Derivatization reagents:
  To improve volatility, increase thermal stability or to achieve a lower limit of detection in gas chromatography
- Prerequisite: quantitative, rapid and reproducible formation of only one derivative
- Halogen atoms inserted by derivatization, e.g., trifluoro-acetates, allow the specific detection in an ECD with the advantage of high sensitivity.
- Specific derivatizations may influence elution orders and fragmentation patterns in a MS
- We provide reagents for
  - acylation
  - alkylation (methylation)
  - silylation
- For 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure

Ordering information

Derivatization method development kits*

Designation	Contents of the kit	REF
Which type of derivatization is suited best for your sample (alkylation, acylation or silylation)?	2 x 1 mL each of TMSH, MSTFA, MBTFA	701952
Acylation kit		
Which is the proper reagent for acylation?	2 x 1 mL each of MBTFA, TFAA, MBHFBA	701950
Alkylation kit		
Which is the proper reagent for methylation?	3 x 1 mL each of TMSH, DMF-DMA	701951
Silylation kit		
Which is the proper reagent for silylation?	2 x 1 mL each of MSTFA, BSTFA, TSIM, MSHFBA	701953

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

Selection guide for derivatization of important functional groups in GC

Function	Method	Derivative	Recommended reagents
alcohols, phenols	silylation	R'O – TMS	BSA, MSTFA, MSHFBA, TSIM, SILYL-2110, SILYL-21, SILYL-1139
R'OH	acylation	R'O – CO – R	TFAA, HFBA, MBTFA, MBHFBA
	alkylation	R'O – R	TMSH
sterically hindered	silylation	R'O – TMS	TSIM, BSTFA, SILYL-991
amines	silylation	R' – NR'' – TMS	BSA, MSTFA, MSHFBA, SILYL-991
primary, secondary	acylation	R' – NR'' – CO – R	TFAA, HFBA, MBTFA, MBHFBA
hydrochlorides	silylation	R' – NR'' – TMS	MSTFA
amides	silylation	not stable	
	acylation	R' – CO – NH – CO – R	TFAA, MBTFA, HFBA, MBHFBA
amino acids	silylation	R' – CH(NH – TMS) – CO – O – TMS	BSA, BSTFA, MSTFA, MSHFBA
	alkylation (a)	R' – CH(NH – CO-R) – CO – O – R	a) MeOH/TMCS, TMSH
	+ acylation (b)		b) TFAA, HFBA, MBTFA, MBHFBA
Carboxylic acids (fatty acids)	silylation	R' – CO – O – TMS susceptible to hydrolysis	BSA, MSTFA, MSHFBA, TMCS, TSIM, SILYL-2110, SILYL-21, Silyl-1139
	alkylation	R' – CO – O – R	DMF-DMA, MeOH/TMCS (1 M), TMSH
salts	silylation	R' – CO – O – TMS susceptible to hydrolysis	TMCS
carbohydrates	silylation		MSTFA, TSIM, HMDS, SILYL-1139
	acylation		TFAA, MBTFA
steroids	silylation		BSA, TSIM
	acylation		TFAA, MBTFA, HFBA, MBHFBA

These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

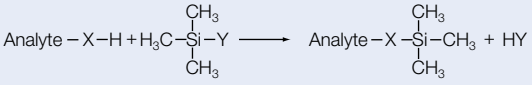
Due to their purpose, derivatization reagents are very reactive substances. For this reason, they should be stored cool and protected from moisture. For easy access with a syringe, our derivatization reagents are supplied in vials with crimp caps (exception DMCS and TMCS with screw closure). Vials with pierced sealing disks have limited stability and should be used soon.

The derivatization procedures can be found on page 367.

Reagents / methods for derivatization

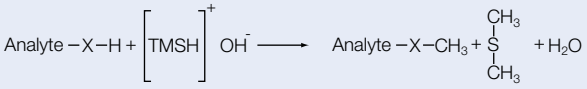
General reaction mechanisms

Silylation



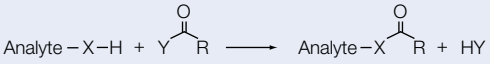
X = e.g., O, S, COO, etc.
Y = rest of silylation reagents

Alkylation (Methylation) · example TMSH



X = e.g., O, S, COO, etc.

Acylation



X = e.g., O, S, NH, etc.
Y = rest of acylation reagents

MACHEREY-NAGEL derivatization reagents for GC

Content of brochure

- Product range for acylation, alkylation and silylation reagents
- Protocols for derivatization
- Diverse tips and hints

Ordner now your derivatization brochure KATEN200144

Acylation reagents

Acyl halides

Key features

- By-product of acylation with acyl halides: corresponding hydrohalic acids excess of reagent and acid have to be removed or trapped by a suitable base (e.g., pyridine)

- Pentafluorobenzoyl chloride
  PFBC: $C_6F_5-CO-Cl$
  M 230.52 g/mol, Bp 158–159 °C (760 mm Hg),
  Density d20°/4° = 1.601

Anhydrides

Key features

- By-products of acylation with anhydrides: corresponding acids excess reagent and the acid formed are to be removed
- Trifluoroacetic acid anhydride TFAA: $CF_3-CO-O-CO-CF_3$
  M 210.04 g/mol, Bp 39.5–40.5 °C (760 mm Hg),
  Density d20°/4° = 1.490

- Heptafluorobutyric acid anhydride
  HFBA: $C_3F_7-CO-O-CO-C_3F_7$
  M 410.06 g/mol, Bp 106–107 °C (760 mm Hg),
  Density d20°/4° = 1.665

Bisacylamides

Key features

- By-products: corresponding neutral acylamides: high volatility
- Easily removed; due to the neutral conditions and their favorable chromatographic characteristics, the removal of surplus bisacylamides and their by-products is often not necessary. Therefore, the sample preparation is much easier.

- *N*-methyl-bis(trifluoroacetamide)
  MBTFA: $CF_3-CO-N(CH_3)-CO-CF_3$
  M 223.08 g/mol, Kp 123–124 °C (760 mm Hg),
  Density d20°/4° = 1.55
- *N*-methyl-bis(heptafluorobutyramide)
  MBHFBA: $C_3F_7-CO-N(CH_3)-CO-C_3F_7$
  M 423.1 g/mol, Kp 165–166 °C (760 mm Hg),
  Density d20°/4° = 1.673

Methods for acylation

Acylation with fluorinated acid anhydrides (TFAA, HFBA)

- Applicable for alcohols, phenols, carboxylic acids, amines, amino acids and steroids, stable derivatives for FID or ECD detection
- Procedure see page 367 or online at *www.mn-net.com/apps*
  TFAA: MN Appl. Nr. 213041
  HFBA: MN Appl. Nr. 213042

Acylation with fluorinated acid amides (MBTFA, MBHFBA)

- Recommended for alcohols, primary and secondary amines as well as for thiols under mild, neutral conditions
- MBTFA also forms very volatile derivatives with carbohydrates [17].
- Procedure see page 367 or online at *www.mn-net.com/apps*
  MBTFA: MN Appl. Nr. 213051
  MBHFBA: MN Appl. Nr. 21305

Ordering information

Acylation reagents*

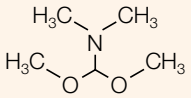
Substance	Packing unit			
	10 x 1 mL	20 x 1 mL	1 x 10 mL	5 x 10 mL
HFBA				
		701110.201	701110.110	701110.510
MBTFA				
		701410.201	701410.110	701410.510
MBHFBA				
	701420.101	701420.201		
PFBC				
	701120.101			
TFAA				
			701130.110	701130.510

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

On request for 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure.

Alkylation / methylation reagents

DMF-DMA *N*,*N*-dimethylformamide dimethylacetal

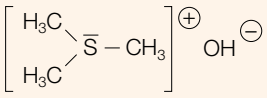


- M 119.17 g/mol,
  Kp 106–107 °C (760 mm Hg),
  Density d20°/4° = 0.897

Key features

- Methylation reagents

TMSH (0.2 mol/L in methanol) Trimethylsulfonium hydroxide



- M 94.06 g/mol

Key features

- Methylation reagents

Methods for alkylation / methylation

Methylation with TMSH

- Suited for free acids, chlorophenoxycarboxylic acids, their salts and derivatives as well as for phenols and chlorophenols [18]
- The great advantage is the simplification of the sample preparation. Lipids or triglycerides can be converted to the corresponding fatty acid methyl esters (FAMEs) by simple transesterification.
- This reaction is very elegant and convenient, because it is only necessary to add the reagent (0.2 mol/L in methanol) to the sample solution. Removal of surplus reagent is not required, since at 250 °C inside the injector of the gas chromatograph, TMSH will pyrolyze solely to volatile methanol and dimethylsulfide. Due to high reactivity, a complete conversion is usually obtained at ambient temperature. Heating (e.g., 10 min at 100 °C) in a closed sample vial may be necessary, however.
- Procedure see page 367 or online at *www.mn-net.com/apps*
  MN Appl. Nr. 213060

Methylation with DMF-DMA

- Applicable for fatty acids, primary amines and (partially) amino acids, under formation of *N*-dimethyl-aminomethylene amino acid methyl esters [19]
- Since DMF-DMA is a poor solvent, it is essential to use a mixture of DMF-DMA with pyridine, THF, acetone (barbiturates) or another solvent.
- Procedure see page 367 or online at *www.mn-net.com/apps*
  MN Appl. Nr. 213070

Methylation with methanol – TMCS (1 M)

- Suited for the esterification of free carboxylic acids and the transesterification of glycerides. Formation of HCl catalyzes the reaction. TMCS, resp. silyl ethers remove the water and thus drive the reaction to completion. The mixture should be freshly prepared.
- Procedure see page 367 or online at *www.mn-net.com/apps*
  MN Appl. Nr. 213080

For GC separation of FAMEs from natural butter fat after derivatization with TMSH see Appl. 201680 at *www.mn-net.com/apps*

Ordering information

Alkylation reagents*

	Packing unit			
Substance	10 x 1 mL	20 x 1 mL	1 x 10 mL	5 x 10 mL
DMF-DMA				
		701430.201	701430.110	
TMSH				
	701520.101	701520.201	701520.110	701520.510

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

On request for 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure.

Reagents / methods for silylation

Silylation reagents

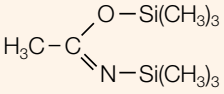
The most common form of silylation in GC is the replacing of active hydrogen atoms with a trimethylsilyl group (TMS derivative). Less frequently, trialkylsilyl groups or dimethylsilyl groups with longer alkyl chains are also in use. The alkylsilyl group increases volatility and enhances thermal stability of the sample.

Silylation can be catalyzed either acidic by addition of TMCS or basic by addition of pyridine or TSIM (e.g., for sterically hindered functionalities like tert. alcohols).

Reactivity of silylation reagents (acc. to M. Donike): TMS amide (e.g., BSA, MSTFA) > TMS amine = TSIM > Enol-O-TMS ether > S-TMS ether > O-TMS ether > TMS-O-TMS

Stability of the TMS derivatives: O-TMS ether > S-TMS ether > Enol-O-TMS ether > TMS amine > TMS amide

BSA *N,O*-bis-trimethylsilyl-acetamide



- M 203.4 g/mol, Bp 71–73 °C (35 mm Hg), Density d20°/4° = 0.832

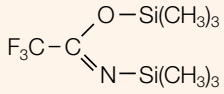
Key features

- Strong silylation reagent
- Not recommended for use with carbohydrates or very low molecular weight compounds
- Good solvent for polar compounds, but frequently used in combination with a solvent (pyridine, DMF etc.) or with other silylation reagents. Dissolved in DMF, BSA is the prime derivatization reagent for phenols.

Recommended application

- Alcohols, amines, carboxylic acids, phenols, steroids, biogenic amines and alkaloids are derivatized to stable TMS derivatives

BSTFA *N,O*-bis-trimethylsilyl-trifluoroacetamide



- M 257.4 g/mol, Bp 40 °C (12 mm Hg), Density d20°/4° = 0.961

Key features

- Powerful trimethylsilyl donor with approx. the same donor strength as the nonfluorinated analog BSA
- Advantage of BSTFA over BSA: greater volatility of its reaction products, particularly useful for GC analysis of low boiling TMS amino acids
- BSTFA is nonpolar (less polar than MSTFA) and can be mixed with acetonitrile for improved solubility. For the silylation of fatty acid amides, hindered hydroxyl groups and other difficult to silylize compounds, e.g., secondary alcohols and amines, we recommend BSTFA + 1 % trimethylchlorosilane (TMCS), available under the designation SILYL-991 (see page 366).

Silylation with BSA, BSTFA or SILYL-991 (BSTFA + 1 % TMCS)

- Procedure see page 367 or online at *www.mn-net.com/apps*

BSA	MN Appl. Nr. 213091
BSTFA	MN Appl. Nr. 213092
SILYL-991	MN Appl. Nr. 213093

Silylation with BSA in combination with other silylation reagents

- Procedure see page 367 or online at *www.mn-net.com/apps*
  MN Appl. Nr. 213100

Ordering information

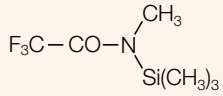
Silylation reagents*

Substance	Packing unit 20 x 1 mL	1 x 10 mL	5 x 10 mL	1 x 50 mL	1 x 100 mL
BSA					
		701210.110	701210.510	701210.150	
BSTFA					
	701220.201	701220.110	701220.510		
SILYL-991 –(BSTFA – TMCS (99:1))					
	701490.201			701490.150	701490.1100

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

On request for 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure.

MSTFA *N*-methyl-*N*-trimethylsilyl-trifluoroacetamide



- M 199.1 g/mol, Bp 70 °C (75 mm Hg), Density d20°/4° = 1.11

Key features

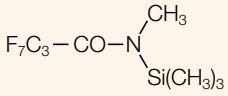
- The most volatile trimethylsilyl amide available, very strong TMS donor which does not cause noticeable FID fouling even during long-time measuring series

Recommended application

- Carboxylic acids, hydroxy and ketocarboxylic acids, amino acids, amines, alcohols, polyalcohols, sugars, mercaptans and similar compounds with active hydrogen atoms. Even amine hydrochlorides can be silylated directly.

- The addition of protic solvents in submolar quantities, e.g., TFA for extremely polar compounds (hydrochlorides) or pyridine for carbohydrates), can improve the already good dissolving power of MSTFA.
- Advantages: complete conversion with high reaction rates, even without a catalyst (1–2 % TMCS or TSIM); the by-product of the reaction (*N*-methyltrifluoroacetamide) shows a high volatility and a short retention time

MSHFBA *N*-methyl-*N*-trimethylsilyl-heptafluorobutyramide



- M 299.1 g/mol, Bp 148 °C (760 mm Hg)

Key features

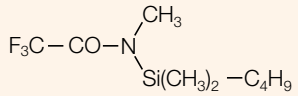
- Similar to MSTFA in reactivity and chromatography
- Either applied alone or in combination with a catalyst (TMCS, TSIM) or another silylation reagent with or without solvent; the by-product *N*-methylheptafluorobutyric amide has a lower retention time than the silylating reagent

Recommended application

- Carboxylic acids, alcohols, phenols, primary and secondary amines and amino acids

- Especially useful for flame ionization detection due to the large ratio of fluorine to silicon of 7:1, since degradation of the surplus MSHFBA does not produce SiO_2 but volatile, non-corrosive silicon compounds

MBDSTFA *N*-methyl-*N*-*tert*-butyldimethylsilyl-trifluoroacetamide



- M 241.3 g/mol, Bp 170 °C (760 mm Hg), Density d20°/4° = 1.121

Key features

- Silylation reagent that donates a *tert*-butyldimethylsilyl group (TBDMS) for derivatizing active hydrogen atoms in hydroxyl, carboxyl and thiol groups as well as primary and secondary amines
- Fast reactions (typically 5–20 min) with high yields (> 96 %), by-products are neutral volatiles
- TBDMS ethers are 10^4 times more stable than the corresponding TMS ethers
- Due to the large protecting group, chromatographic retention times are longer. This may have a beneficial impact on some separations. The high concentration of M^+-57 ions is an interesting topic for GC/MS.

Reagents / methods for silylation

Silylation with MSTFA, MSHFBA or MBDSTFA

- Procedure see page 367 or online at *www.mn-net.com/apps*
  MSTFA MN Appl. Nr. 213111 · MSHFBA MN Appl. Nr. 213112 · MBDSTFA MN Appl. Nr. 213113

Ordering information

Silylation reagents*

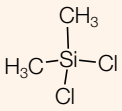
	Packing unit							
Substance	10 x 1 mL	20 x 1 mL	1 x 10 mL	5 x 10 mL	1 x 100 mL	6 x 50 mL	6 x 100 mL	12 x 100 mL
MSTFA								
		701270.201	701270.110	701270.510	701270.1100	701270.650	701270.6100	701270.12100
MSHFBA								
		701260.201	701260.110	701260.510	701260.1100		701260.6100	
MBDSTFA								
	701440.101	701440.201						

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

On request for 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure.

Ultrapure derivatization reagents for acylation, alkylation and silylation.

DMCS Dimethyldichlorosilane

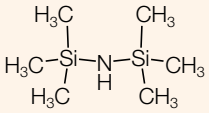


- M 129.06 g/mol,
  Bp 70 °C (760 mm Hg),
  Density d20°/4° = 1.07

Key features

- Used to form dimethylsilyl (DMS) derivatives
- DMS derivatives are much more susceptible to hydrolysis than TMS derivatives, it is therefore vital to have strictly anhydrous conditions during the conversion.

HMDS Hexamethyldisilazane

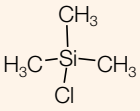


- M 161.4 g/mol,
  Bp 126 °C (760 mm Hg),
  Density d20°/4° = 0.7742

Key features

- Weak TMS donor; used as a sole reagent, it is slow and not very effective.
- Aprotic solvents like acetonitrile, pyridine, dimethylformamide, carbon disulfide and dimethylacetamide recommend themselves for use with HMDS.
- With catalytic quantities, e.g., 1 % of, or as a mixture with TMCS (2:1, v/v; SILYL-21 and SILYL-2110) it is perfectly suited for a quick and quantitative trimethylsilylation of organic compounds.

TMCS Trimethylchlorosilane

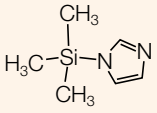


- M 108.7 g/mol,
  Bp 57 °C (760 mm Hg),
  Density d20°/4° = 0.8580

Key features

- Often used as a catalyst with other trimethylsilyl reagents
- As a sole reagent, it can be used to prepare TMS derivatives of organic acids.

TSIM *N*-trimethylsilyl-imidazole



- M 140.3 g/mol,
  Bp 94–96 °C (760 mm Hg),
  Density d20°/4° = 0.961

Key features

- Strongest hydroxyl silylator
- It is remarkable that TSIM reacts quickly and smooth with hydroxyl (even tert. OH) and carboxyl groups, but not with amines. Hence it is especially suited for multiple derivatizations, when compounds with various functional groups are to be derivatized in different ways (e.g., -O-TMS, -*N*-HFB derivatives of catecholamines).

Recommended application

- Alcohols, phenols, organic acids, steroids, hormones, glycols, nucleotides, narcotics
- Reagent of choice for carbohydrates and most steroids (even strongly hindered steroids)

Silylation with TSIM or SILYL-1139 (TSIM – pyridine 11:39)

- Procedure see page 367 or online at *www.mn-net.com/apps*

TSIM: MN Appl. Nr. 213121
SILYL-1139: MN Appl. Nr. 213122

Reagents / methods for silylation

Ordering information

Silylation reagents*

	Packing unit			
Substance	20 x 1 mL	1 x 10 mL	5 x 10 mL	6 x 50 mL
DMCS				
				701230.650
HMDS				
			701240.510	701240.650
TMCS				
	701280.201			701280.650
TSIM				
	701310.201	701310.110	701310.510	

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

On request for 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure.

Ordering information

Reagent mixtures for silylation*

		Packing unit				
Mixture	Composition	20 x 1 mL	1 x 10 mL	5 x 10 mL	1 x 50 mL	1 x 100 mL
SILYL-271						
	BSA - HMDS - TSIM (2:7:1)	701450.201	701450.110	701450.510		
SILYL-1139						
	TSIM - Pyridine (11:39)	701460.201				
SILYL-21						
	HMDS - TMCS (2:1)	701470.201				
SILYL-2110						
	HMDS - TMCS - Pyridine (2:1:10)	701480.201				
SILYL-991						
	BSTFA - TMCS (99:1)	701490.201			701490.150	701490.1100

* These products contain harmful substances which must be specially labeled as hazardous. For detailed information please see SDS.

On request for 1 x 10 mL, 1 x 50 mL and 6 x 50 mL also available with screw closure.

Due to their purpose, derivatization reagents are very reactive substances. For this reason, they should be stored cool and protected from moisture. For easy access with a syringe, our derivatization reagents are supplied in vials with crimp caps (exception DMCS and TMCS with screw closure). Vials with pierced sealing disks have limited stability and should be used soon.

Silylation with SILYL-21 or SILYL-2110

- Recommended applications: sugars, glycols, sterically unhindered alcohols, carboxylic acids, acids in urine, hydroxy fatty acids, nucleotides, steroids, vitamin D, xanthone derivatives
- Procedure see page 367 or online at *www.mn-net.com/apps*
  SILYL-21 MN Appl. Nr. 213131
  SILYL-2110 MN Appl. Nr. 213132

O-trimethylsilylation with MSTFA followed by *N*-trifluoroacetylation with MBTF

- Procedure see page 367 or online at *www.mn-net.com/apps*
  MSTFA / MBTFA MN Appl. Nr. 213140

Acylation

with fluorinated acid anhydrides · TFAA MN Appl. No. 213041 · HFBA MN Appl. No. 213042

Dissolve 0.1 to 1 mg sample in 0.1 mL solvent, add 0.1 mL of the anhydride and heat to 60–70 °C for 1–2 h. If the sample needs not be concentrated prior to the analysis and if there is no danger of catalytically induced side reactions, pyridine is used as solvent. The reaction solution can be injected directly into the gas chromatograph. Otherwise, use a volatile solvent and evaporate solvent, excess reagent and free acid in a stream of nitrogen. Dissolve residue in 50 µL hexane, chloroform etc. and inject aliquot portions.

with fluorinated acid amides · MBTFA MN Appl. No. 213051 · MBHFBA MN Appl. No. 213052

Add 0.5 mL MBTFA or MBHFBA to about 2 mg sample. If there is no reaction at ambient temperature, heat the reaction mixture to 120 °C. Compounds difficult to dissolve, can be trifluoroacetylated in suitable solvent mixtures. It is recommended to use a ratio of solvent to MBTFA or MBHFBA of 4:1. The reaction mixture is chromatographed directly.

Alkylation (Methylation)

with TMSH · MN Appl. No. 213060

Dissolve 100 mg sample (e.g., butter) in 5 mL of a solvent (e.g., *tert*.-butyl methyl ether). Add 50 µL reagent to 100 µL of this solution. The mixture is injected directly. The temperature of the injector must be at least 250 °C.

with DMF-DMA · MN Appl. No. 213070

Add 1 mL of a mixture of DMF-DMA and pyridine (1:1) to 1–50 mg fatty acids. The sample can be injected as soon as a clear solution has formed. It is recommended, however, to heat the solution to 60–100 °C for 10–15 min.

with methanol – TMCS · MN Appl. No. 213080

Add 1 mL methanol – TMCS to about 50 mg carboxylic acid or glyceride and heat. Then evaporate in a stream of nitrogen and dissolve again for injection in, e.g., *n*-heptane.

Silylation

with BSA, BSTFA oder SILYL-991 (BSTFA + 1 % TMCS)
BSA MN Appl. No. 213091 · BSTFA MN Appl. No. 213092 SILYL-991 MN Appl. No. 213093

Add 0.5 mL of the silylation reagent to 1–10 mg sample; if necessary, add some solvent (normally pyridine or DMF [dimethylformamide]). Heat to 60–80 °C for 20 min to increase the reaction rate. 1–2 drops of TMCS (trimethylchlorosilane) or TSIM will also speed up the reaction.

with BSA in combination with other silylation reagents · MN Appl. No. 213100

BSA alone silylates all sterically unhindered hydroxyl groups of the steroid skeleton; addition of TMCS will enable reaction of moderately hindered OH groups (reaction time 3–6 h at 60 °C). After addition of TSIM even strongly hindered hydroxyl groups will react (reaction time 6–24 h at 60 °C).

with MSTFA, MSHFBA or MBDSTFA
MSTFA MN Appl. No. 213111 · MSHFBA MN Appl. No. 213112 · MBDSTFA MN Appl. No. 213113

Dissolve 10–15 mg sample in 0.8 mL solvent, then add 0.2 mL of the silylation reagent. The reaction mixture can be heated to 60–70 °C for up to 1 h and can be analyzed directly. If TFA is used as a solvent, proceed as follows [20]: dissolve 1–2 mg sample in 100 µL TFA. Dropwise add 0.9 mL of the silylating reagent. After cooling the sample can be chromatographed directly.

with TSIM or SILYL-1139 (TSIM – pyridine 11:39) · TSIM MN Appl. No. 213121 · SILYL-1139 MN Appl. No. 213122

Dissolve 10–15 mg sample in 0.8 mL solvent, then add 0.2 mL of the silylation reagent. The reaction mixture can be heated to 60–70 °C for up to 1 hour and can be analyzed directly. Recommended solvent pyridine. When using SILYL-1139, the presence of water does not interfere.

with SILYL-21 or SILYL-2110 · SILYL-21 MN Appl. No. 213131 · SILYL-2110 MN Appl. No. 213132

Carefully add SILYL-21 or SILYL-2110 to 1–10 mg of the sample. Precipitated ammonium chloride does not interfere. If the sample should not dissolve within 5 min, heat to 75–85 °C. If no mutarotation is to be expected, you may dissolve the sugar in warm pyridine first and then add the silylation reagent. In some cases it may be advantageous to use a different solvent instead of pyridine. For derivatization of 3-ketosteroids we recommend to use DMF (dimethylformamide)

O-trimethylsilylation with MSTFA followed by *N*-trifluoroacetylation with MBTFA · MN Appl. No. 213140

Completely silylate 2 mg of the sample with 0.3 mL MSTFA, e.g., as described on page 363. After addition of 0.3 mL MBTFA the *N*-trimethylsilyl group is replaced by the *N*-trifluoroacetyl group. The mixture can be analyzed directly.