B Longitudinal diffusion term of the van Deemter and Golay equations.

β Column phase ratio. The column volume occupied by mobile (gas) phase relative to the volume occupied by stationary phase. In open-tubular columns: $\beta = [r_c - 2d_f]/[2d_f] \cong r_c/2d_f$.

c_M, c_S Solute concentrations in mobile and stationary phases, respectively.

C Resistance to mass transfer (or mass transfer) in the van Deemter (or Golay) equations; C_M and C_S denote mass transfer from mobile to stationary and from stationary to mobile phases, respectively.

d_c Inner diameter of the column. Both mm and μm are commonly used. The latter, while consistent with the units used for d_f, implies three significant figures, which is rarely true.

d_f Thickness of the stationary phase film, usually in μm.

D Diffusivity; D_M and D_S denote solute diffusivities in the mobile and stationary phases, respectively; usually given in $cm^2\ s^{-1}$.

F Volumetric flow of the mobile phase, usually in $cm^3\ min^{-1}$. Many practical chromatographers assume equivalency with (and hence employ) $mL\ min^{-1}$.

FID Flame ionization detector.

GC-MS The combination of gas chromatography and mass spectrometry, usually a single integrated unit in which fractions separated by GC are sequentially introduced to the MS.

H Length equivalent to one theoretical plate (height equivalent to a theoretical plate): $H = L/N$. When measured at u_{opt}, the result is termed H_{min}.

k Solute retention factor (formerly partition ratio). Ratio of the amounts of a solute (or time spent) in stationary and mobile phases, respectively. Because all solutes spend t_M time in the mobile phase, $k = [t_R - t_M]/t_M$, and $k = t'_R/t_M$.

K_c Distribution constant. Formerly K_D. Ratio of solute concentrations in stationary and mobile phases, respectively: $K_c = c_S/c_M$.

L Length of the column, usually expressed in metres for column length, in cm for the determination of $\bar{u}$, and in mm for the determination of H.

N Theoretical plate number; $N = [t_R/\sigma]^2$, where σ is the standard deviation of the peak.

N_{req} Number of theoretical plates required to separate two solutes of a given alpha and given retention factors to a given degree of resolution: $N_{req} = 16R_s^2[(k+1)/k]^2[\alpha/(\alpha-1)]^2$.

o.d. Outer diameter of the column.

r_c Inside radius of the column.

R_s Peak resolution. A measure of separation as evidenced by both the distance between the peak maxima and by the peak widths. ASTM and IUPAC definitions are based on w_b (peak width at base) measurements, which require extrapolation. If peaks are assumed to be Gaussian, then $R_s = 1.18[t_{R(B)} - t_{R(A)}]/[w_{h(A)} + w_{h(B)}]$.

σ Standard deviation of a Gaussian peak.

t_M Gas hold-up time. The time (or distance) required for a nonretained substance (e.g. mobile phase) to transit the column.

t_R Retention time. The time (or distance) from the point of injection to the peak maximum.

t'_R Adjusted retention time. Equivalent to the residence time in stationary phase; difference of the solute retention time and the gas hold-up time: $t'_R = t_R - t_M$.

$\bar{u}$ Average linear velocity of the mobile (gas) phase: $\bar{u}\ (cm\ s^{-1}) = L\ (cm)/t\ (s)$.

V Volume. V_M and V_S represent volumes of the mobile and stationary phases, respectively.

w_b Peak width at base. Determined by measuring the length of baseline defined by intercepts extrapolated from the points of inflection of the peak, and equivalent to four standard deviations in a Gaussian peak.

w_h Peak width at half-height. Measured across the peak halfway between baseline and peak maximum, this can be measured directly without extrapolation, and is equal to 2.35 standard deviations in a Gaussian peak.

Derivatization

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Introduction

Gas chromatography (GC), the longest established instrumental chromatographic technique, dominated the separation field from the early 1950s until the mid-1970s when high performance liquid chromatography (HPLC) became a competitive technique. During this 20-year period, considerable effort was expended in developing procedures to make compounds sufficiently volatile, more thermally stable and less polar so that they would be more amenable to GC analysis. Such efforts were aimed almost

exclusively at removal of active hydrogen atoms(s) from protonic functional groups by the action of a suitable reagent, giving rise to a derivative with the hydrogen atoms substituted by less active functional groups.

Development of a particular derivatization method requires a good knowledge of organic chemistry, taking into consideration as many reaction mechanisms as possible. This is particularly true for the derivatization of protein amino acids, where so many different chemical groups are involved, such that a remark has been made about having here 'the whole of Beilstein'! The history of their more or less successful derivatization can be found in the book *Amino Acid Analysis by Gas Chromatography* (see Further Reading).

In general, the nature of the compounds to be analysed and their chemical properties govern the choice of the particular chemical treatment. This does not mean, however, that the reactions used on a macro-scale by organic chemists can be automatically adopted to the scale of microlitre volumes. The recent discovery of chloroformate-induced esterification of carboxylic acids is a good example of this. Over time, many derivatization methods have become more or less obsolete as their original usefulness was determined by a lack of alternative methods for the determination of minute amounts of some analytes, especially those in biological fluids. Until the discovery of immunoassay (radioimmunoassay, RIA, and enzyme immunoassay, EIA) and the development of specific and sensitive HPLC detectors, the electron-capture detector (ECD) in GC was the only way to reach picomole concentration levels. At that time, therefore, there was considerable interest in the conversion of analytes into perhalogenated products with a correspondingly high ECD response. Some of these methods or principles still persist; others do not. A comprehensive list of various chemical treatments can be found in the *Handbook of Derivatives for Chromatography* by Blau and Halket (see Further Reading). Some of the older useful methods and recent novel discoveries will be discussed in more detail below (see also the reviews by Hušek and Wells listed in the Further Reading section).

Esterification

Carboxylic acid groups usually require treatment with a group-oriented reagent that will not react in most cases with any other protonic groups that may be present. The choice of treatment depends on what class of acidic compounds – with or without extra reactive groups in the molecule – is to be esterified and what kind of detection (ECD or flame ionization detection, FID) is required.

Esterification with Acidified Alcohols

For analytical work, esterification with methanol through to isoamyl alcohol is best done in the presence of a volatile catalyst such as hydrogen chloride, thionyl chloride or acetyl chloride, which can be readily removed together with any excess alcohol. Fatty acids are often methylated by a short boiling with BF_3/methanol, and this catalyst has proved to be effective even for transesterification of acylglycerols (neutral lipids are, however, most easily saponified using sodium or potassium hydroxide in methanol). Higher alcohols, most often *n*-butanol and isobutanol, have been used for HCl-catalysed esterification of amino acids in two steps (with lower alcohols, evaporative losses of the lower mass members occur). For effective conversion, heating at or above 100°C is required.

Esterification via Pyrolytic Methylation

Strongly basic quaternary salts of ammonia, e.g. tetramethyl or trimethylphenylammonium hydroxide in methanol, when co-injected with fatty acids into the heated inlet of a GC, convert the acids into quaternary salts that are immediately pyrolysed into methyl esters and swept onto the analytical column. However, this rapid procedure is not recommended for polyunsaturated fatty acids because isomerization of the double bonds may occur owing to the high inlet temperature and alkalinity of the reagents. It has been reported that tetraalkylammonium fluorides, cyanides or acetates frequently offer considerable advantages over the hydroxides in terms of derivatization selectivity without compromising derivatization efficiency. Regarding on-column benzylation reagents, the 3,5-bis(trifluoromethyl)benzyldimethylphenylammonium fluoride and some related compounds have been shown to be very useful new derivatization reagents with a variety of uses (see the review by Wells in the Further Reading section). Dimethylformamide dimethylacetal $(CH_3)_2NCH(OCH_3)_2$ and its higher alkyl analogues have also been employed as hot inlet esterification agents. Hopes of being able to use them for one-step derivatization of amino acids have not been fulfilled, however.

Esterification with Diazoalkanes

Diazomethane, a yellow gas normally used as a solution in ether, readily esterifies fatty acids in the presence of methanol at room temperature and excess reagent can easily be removed by evaporation. However, the gas is highly carcinogenic and unstable, partially reacting with double bonds, carbonyl, phenolic and hydroxyl groups. This method, once popular, is now the exception. The same is true for the

higher analogues diazoethane and phenyldiazomethane (used to make benzyl esters). The recent use of commercially available trimethylsilyldiazomethane (solution in hexane) has proved to be useful in some applications.

Esterification via Carbodiimide-Induced Coupling

The carbodiimide-coupled esterification of carboxylic acids is a well-known reaction in organic chemistry. *N*,*N*′-Dialkylcarbodiimides act as water scavengers, promoting condensation of an acid with an alcohol while being transformed into an *N*,*N*′-dialkylurea (from X–N=C=N–X to X–NHCONH–X, where X is an alkyl radical). For example, diisopropylcarbodiimide (DIC) was used for the esterification of some aromatic acids with hexafluoroisopropanol. A concentration of 0.1–0.15% DIC and the alcohol in hexane was found to be sufficient to esterify within 1 min.

Esterification with Alkyl Halides

Alkyl (aryl) iodides, bromides (less often chlorides) have been used for treatment of potassium, *t*-amines, tetraalkylammonium or 'crown' ether salts of acids in a suitable organic solvent such as acetonitrile, acetone or methanol. Corresponding alkyl/aryl esters are formed without heating the sample at optimum conditions in high yields. It has been reported by Wells and co-workers (see Further Reading) that macroporous quaternary ammonium anion exchange resins are a very effective support matrix for the methylation of strongly acidic organic analytes with methyl iodide in either supercritical carbon dioxide or acetonitrile. The isolation and determination of simple volatile aliphatic acids from urine by trapping on ion exchange resin followed by simultaneous derivatization with pentafluorobenzyl bromide (PFBBr) and extraction with supercritical carbon dioxide has also been reported. The electron-capturing pentafluorobenzyl (PFB) esters have become increasingly popular for GC-ECD analysis of fatty acids regardless of chain length. Conversion to PFB esters has been reported to succeed in the presence of acetonitrile and diisopropylethylamine in 10 min.

Silylation

A wide range of highly reactive and specific reagents for nearly every application with a trimethylsilyl (TMS) or the increasingly popular *t*-butyldimethylsilyl (TBDMS) donor is now available. These reagents, which are of general utility, will be discussed in a separate section. Some of them can convert the carboxylic acid group into the TMS ester ($RCOO–Si(CH_3)_3$) practically instantaneously and GC analysis can be done by direct injection of the reaction mixture. Reagents with a TBDMS donor require longer reaction times but provide hydrolytically more stable derivatives. Under controlled conditions, with mild silylating agents, e.g. hexamethyl disilazane (HMDS), only the carboxylic acid group will be esterified. Silylation is the preferred way for treating dicarboxylic acids or polycarboxylic compounds. Haloalkylsilylation reagents have been used for sensitive ECD detection but they are expensive and less popular than they once were.

Derivatization by Alcoholysis of the Intermediate Mixed Anhydrides

Haloalkyl anhydrides, especially the perfluorinated ones of acetic, propionic and butyric acids, are able to promote condensation reactions with an alcohol (see **Table 1** for the reagents and abbreviations), when in a molar excess over the alcohol. TCE, TFE or HFIP in combination with TFA or HFBA have often been used to attain higher ECD response. Heating is usually employed without the catalytic presence of an organic base. This approach will be mentioned again in the next section.

At the beginning of the 1990s, chloroformates with the simplest alkyl groups, i.e. methyl and ethyl chloroformate (MCF and ECF), were shown to act as exceptionally rapid esterification reagents. The catalytic presence of pyridine has been found to be a prerequisite for ester formation; water can be, or for some applications should be, a constituent of the medium, together with an alcohol and commonly acetonitrile also. This promising treatment has a broader utility and will be discussed further. The reaction mechanism is based, as in the first case, on alcoholysis of a mixed anhydride intermediate. The advantageous use of this approach in comparison with the former can be seen from **Table 2**.

Acylation/Alkylation

Reagents treated in this section have proved to be especially suitable for handling the various nitrogen protic groups for which silylation with TMS donors is clearly inferior due to the lability of the N–Si bond. A comprehensive review on the derivatization of amines for GC analysis has been published by Kataoka (see Further Reading).

The perfluorinated anhydrides, despite their much higher molecular weight, yield derivatives of high volatility since interaction between the perfluoroalkane chain and a nonfluorinated stationary phase is substantially weakened. The retention of perfluoroalkyl(acyl) derivatives is, therefore, often markedly less

Table 1 Electrophoric reagents frequently used for esterification, acylation and alkylation

Haloalkyl(*acyl*) *group*	*Matrix*	*Formula*	*b.p.* (°C)	*Abbreviation*
2-Chloro-	Ethanol	$ClCH_2CH_2OH$	129	2CE
2,2,2-Trichloro-	Ethanol	CCl_3CH_2OH	151	TCE
	Ethyl chloroformate	CCl_3CH_2OCOCl	171	TCECF
2,2,2-Trifluoro-	Ethanol	CF_3CH_2OH	78	TFE
	Acetic anhydride	$(CF_3CO)_2O$	40	TFAA
Chlorodifluoro-	Acetic anhydride	$(CF_2ClCO)_2O$	96	CDFAA
1,1,1,3,3,3-Hexafluoro-	Isopropanol	$(CF_3)_2CHOH$	59	HFIP
2,2,3,3,3-Pentafluoro-	Propanol	$C_2F_5CH_2OH$	81	PFP
	Propionic anhydride	$(C_2F_5CO)_2O$	96	PFPA
2,2,3,3,4,4,4-Heptafluoro-	Butanol	$C_3F_7CH_2OH$	96	HFB
	Butyric anhydride	$(C_3F_7CO)_2O$	108	HFBA
	Butyryl chloride	C_3F_7COCl	39	HFB-Cl
Pentafluorobenzoyl	Chloride	$C_6F_5CH_2COCl$	158	PFB-Cl
Pentafluorobenzyl	Bromide	$C_6F_5CH_2Br$	174	PFBBr
	Chloroformate	$C_6F_5CH_2OCOCl$		PFBCF
	Hydroxylamine	$C_6F_5CH_2ONH_2$		PFBHA
	Aldehyde	C_6F_5CHO	165	PFBA

than that of their hydrocarbon analogues. Another reason for their popularity is the high sensitivity to ECD, which increases rapidly with increase in F substitution. The response can be further augmented by incorporation of Cl, Br or I atoms into the molecule but the volatility of such derivatives declines rapidly and reagents carrying more than three Cl atoms are unsuitable for derivatization of higher mass analytes.

Treatment of Amino and/or Hydroxyl Groups

Reactive anhydrides listed in Table 1 are frequently employed for this purpose. Acetic anhydride, which has the additional advantage of reacting in aqueous media, has proved its usefulness for esterification of phenolic hydroxyl groups. However, its lower reactivity and the lower volatility of the (per)acetylated forms prevents its wider use.

Acylation of amino acid butyl or isobutyl esters with TFA or HFBA carried out at 100°C or more, usually in the presence of a solvent such as dichloromethane after evaporation of the first esterification medium, is one of the best-established procedures of the 1960s and 1970s. All protein amino acids, including arginine, which is the most difficult amino acid to derivatize and determine by GC, have been successfully analysed and quantitated. Experience gained in the derivatization of amines, alcohols and acids is valuable for procedures for amino acids. A study of these approaches affords a basic knowledge of commonly occurring derivatization problems.

Acid halides have been employed for acylation less often. Except for PFB-Cl, which has even been used for acylation of amino acid isobutyl esters, the use of other acid chlorides is rather rare. However, surprisingly good results have been reported for catecholamines treated with HFB-Cl, which results in ready acylation of the phenolic, amino and alcoholic groups of analytes in an aqueous buffer. Unlike other chlorides, which are almost explosively reactive with water, HFB-Cl appears to be more stable towards hydrolysis. Many applications, aimed at the trace analysis of amines, phenols and alcohols with ECD by acylation with haloanhydrides are outside the

Table 2 Comparison of the reaction conditions for HFBA- and the ECF-induced esterification of carboxylic acids

	HFBA-catalysed[a]		*ECF-catalysed*[b]	
Medium	Chloroform		Chloroform	Water-acetonitrile (1 : 1)
Alcohol	TCE (1–10%)	EtOH (20%)	EtOH (1%)	EtOH (30%)
Reagent	HFBA (six-fold volume)		ECF (1%)	ECF (5%)
Base	None	Pyridine (25%)	Pyridine (2%)	Pyridine (8%)
Reaction time	30 min	4 min (boiling)	Few seconds	
Yield	Not given		> 90%	
Extraction	Chloroform versus aq. HCl and NaOH		Hexane versus aq. $NaHCO_3$	

[a]Edman DC and Brooks JB (1983) Gas-liquid chromatography—frequency pulse-modulated electron-capture detection in the diagnosis of infectious diseases. *Journal of Chromatography* 274: 1–25.

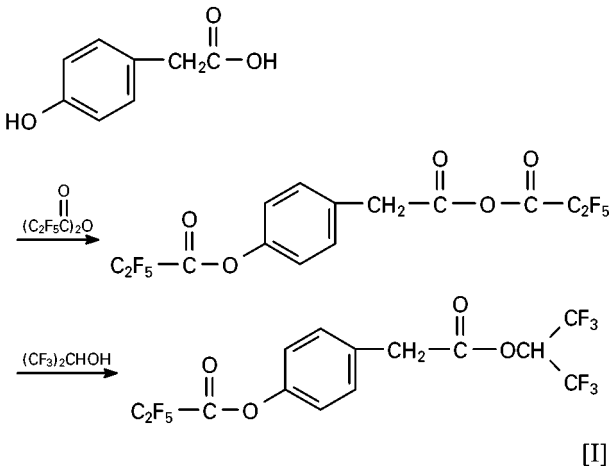
[b]Hušek P, Rijks JA, Leclercq PA and Cramers CA (1990) Fast esterification of fatty acids with alkyl chloroformates. Optimization and application in gas chromatography. *Journal of High Resolution Chromatography* 13: 633–638.

scope of this article; for details the reader should consult the Blau and Halket book or the papers by Poole (see Further Reading).

The amino group is best derivatized in aqueous media with chloroformates. Catecholamines are treated with MCF; if the alcoholic OH group is present it is silylated or acetylated in addition. During this step the MCF-alkylated phenolic hydroxyls reconvert to *O*-TMS or *O*-acetyl esters and only the amino group retains the alkyl group from the MCF. On well-deactivated capillaries the analysis succeeds even without this extra treatment. When tertiary amines are treated with chloroformates, the smallest alkyl group attached to the nitrogen undergoes a displacement and the amine is thus transformed to a carbamate. Hydrolysis of the latter yields a secondary amine, which is then exposed for further derivatization.

Concurrent Treatment Involving Carboxylic Groups

As already mentioned, the perfluorinated anhydrides TFAA, HFBA and less often the more expensive PFPA, can promote esterification of carboxylic groups provided that they are in a molar excess over the haloalcohol present. It is logical to suppose that even polyfunctional acids might be effectively treated, since side chain groups are acylated smoothly. This has been confirmed experimentally by treating hydroxyphenolic acids, bile acids and even amino acids, the carboxylic and side chain groups of which are esterified and acylated simultaneously. It is assumed that the reaction proceeds in two steps, i.e. by the formation of mixed anhydride intermediate that is subsequently alcoholysed to the ester, as shown by *p*-hydroxyphenylacetic acid treated with PFPA/HFIP (reaction [I]). Derivatization of a number of carboxylic acids has been achieved by treatment with TFAA and HFB or TFE in the presence of an organic base with heating to 60°C for 30–40 min.

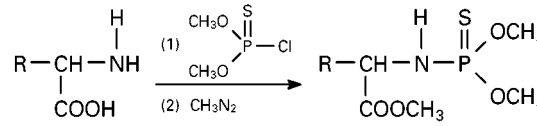


[I]

Alkylation Preceding the Carboxyl Treatment

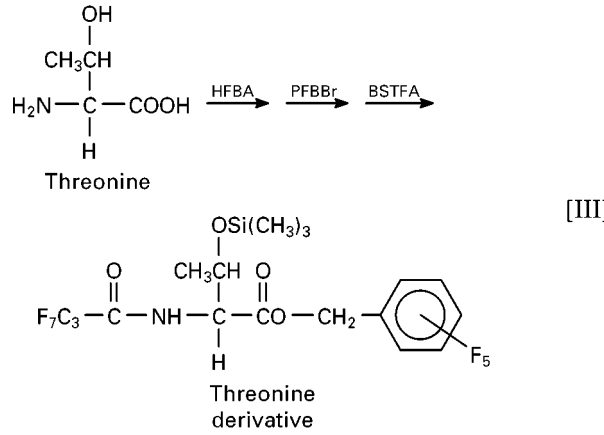
This procedure deals with amino acids in the reverse order – first the amino group, including any side chains, is derivatized, and then the carboxyl group is methylated or silylated. The unique ability of chloroformates to react rapidly with analytes in aqueous media is utilized in a procedure employing isobutyl chloroformate (IBCF) followed by methylation with diazomethane. More than 50 amino acids (with the exception of arginine) have been analysed in this way as their *N*-isobutyloxycarbonyl (IBOC) methyl esters.

Alternatively, instead of methylation, the carboxylic group can be treated with a TBDMS donor and many amino acids have been analysed as *N*(*O*,*S*)-IBOC TBDMS esters. An ECD-oriented procedure was based on derivatization of the amino groups with dimethylchlorthiophosphate, followed by methylation (reaction [II]).



[II]

Likewise a three-stage treatment involving acylation of the amino groups with HFBA, extractive alkylation with PFBBr (for the carboxyl groups) followed by BSTFA silylation of aliphatic hydroxyl groups served the same purpose. The derivatization of threonine is an example of this procedure (reaction [III]). This scheme may seem unnecessarily complicated but the treatment is rapid and conditions are mild as each group undergoes its respective derivatization. In general the use of one reagent to react with different groups requires more drastic conditions.



[III]

Oximation of keto groups is one way to prevent isomerization (and/or enol formation) of keto acids before further derivatization by, for example, silylation. The keto group reacts with hydroxyl, methoxy

or ethoxyamine as follows:

$$> C=O + H_2N\text{–}OR \rightarrow C=N\text{–}OR \qquad [IV]$$

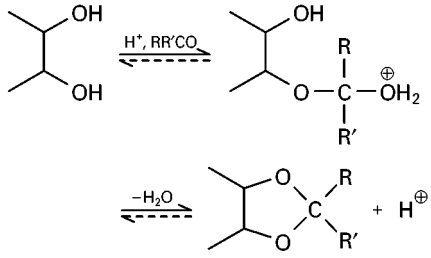
If one wishes to shift the derivatization products of simple aliphatic keto acids to products with greater retention times, then a correspondingly higher boiling reagent PFBHA (see Table 1) can be employed.

Cyclization

Some reagents selectively react with two protonic groups to form a heterocyclic compound with five, six or seven atoms, i.e. the groups to be treated are in positions 1,2, 1,3 or 1,4 on an aliphatic chain or 1,2 on an aromatic ring (see the review by Poole and Zlatkis listed in the Further Reading).

Acetals/ketals

This kind of cyclization is often used to provide protection for *cis*-diols and thiols. It is based on the reaction of aldehydes or ketones with 1,2-diols in the presence of an acid catalyst and proceeds via the formation of a hemiketal that is further rearranged to the dioxolane product (called acetonide or isopropylidene when acetone is used as the ketone; see reaction [V]).

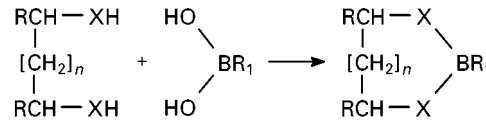


[V]

Acetonide derivatives are prepared, e.g. from α-monoacylglycerols (β-isomers do not react), and separated by GC. Corticosteroids with *cis*-C-20,21- or C-17,20-dihydroxy groups form acetonides under mild conditions and the products are suitable for GC analysis. The acetonide group is also stable under conditions of further treatment such as silylation or acetylation. The reaction is specific to *cis*-diols since the *trans*-C-20,21 diol does not form an acetonide. Siliconides are also formed by a similar mechanism when dimethylchlorosilane is used in pyridine, but multiproduct formation and instability of the derivatives has prevented wider use.

Boronates

Many diols are best handled with boronic acids. Alkyl boronates were used extensively in the 1970s since they exhibit a broad ability to cyclize and are able to bridge diols up to a 1,4 position. The cyclic products (reaction [VI]) are stable enough to be analysed by GC and may even be made in the GC inlet.

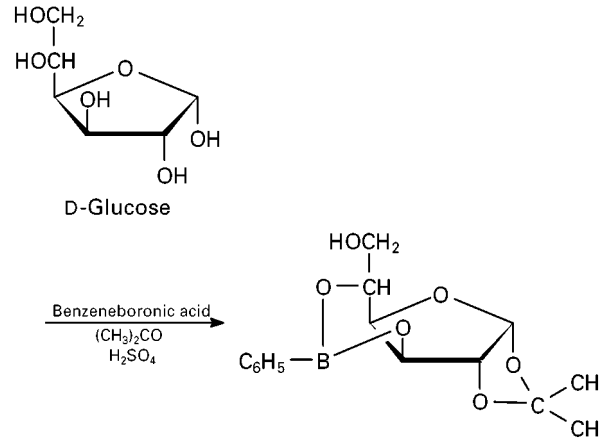


[VI]

X = O, N, S, CO_2
n = 0, 1, 2

Cyclic boronate

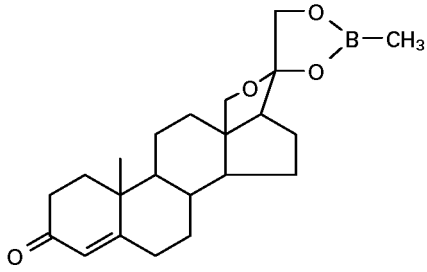
However, hydrolytic stability is not good and the products of reaction with a single OH group cannot be analysed. This can cause problems with, for example, steroids containing an additional alcohol group or groups next to the diol. The required additional treatment (acetylation, silylation) often results in unwanted by-products. Various alkyl groups, such as *t*-butyl, have been investigated with a view to improving the stability of the product, but the results have not always been as expected. The *n*-butaneboronates offer a convenient compromise between volatility and stability. For ECD, 2,4-dichloro- and 3,5-bis(trifluoromethyl)benzeneboronic acid have proved to be the best, since PFB-boronates are unstable to hydrolysis. Some useful applications include treatment of monosaccharides with benzeneboronic acid in acetone to give mixed acetonide–boronate products, as shown in reaction [VII]).



[VII]

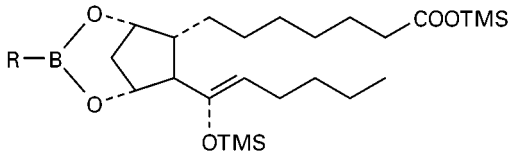
The action of methaneboronate on the corticosteroid (structure [VIII]) leads to an interesting rearrangement in the side chain and the selective reaction of the *cis*-diol group of prostaglandin F allows it to be distinguished (after silylation) from the structure with a *trans*-diol group, which does not react (structure [IX]). Boronates as reagents for GC analysis

are now rather ancient history but they do represent a considerable amount of successful past effort.



Methaneboronate of 18-hydroxy-11-deoxycorticosterone

[VIII]



Prostaglandin $F_{1\alpha}$ cyclic boronate TMS ether TMS ester derivative

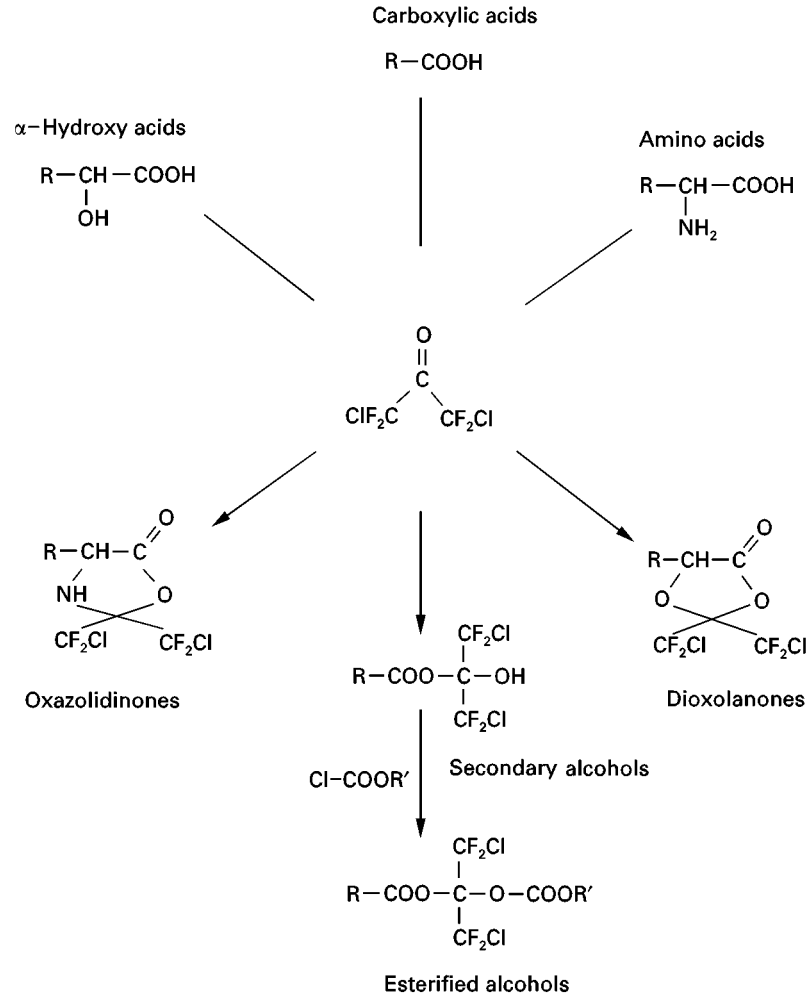
[IX]

Oxazolidones

The conditions for the condensation of amino acids with 1,3-dichlorotetrafluoroacetone (DCTFA) were established in the early 1980s, but before the method could establish its worth HPLC became the preferred technique and the reagent disappeared from the catalogues of all the major suppliers.

Substitution of halogens into the acetone molecule enhances the acidic character of the carbonyl group and promotes formation of stable adducts that are not otherwise obtained with aliphatic ketones. A scheme for some reactions with DCTFA is shown in scheme [X].

The double-step treatment with DCTFA followed by HFBA in the same aprotic medium, together with the subsequent analysis by capillary GC, gave the most rapid analysis of the protein amino acids, including arginine, at the time the method was developed. With an ECD, femtomole levels are easily attained. The method was originally developed for the derivatization of thyroid hormones with three to four iodine atoms in the molecule. The derivatized

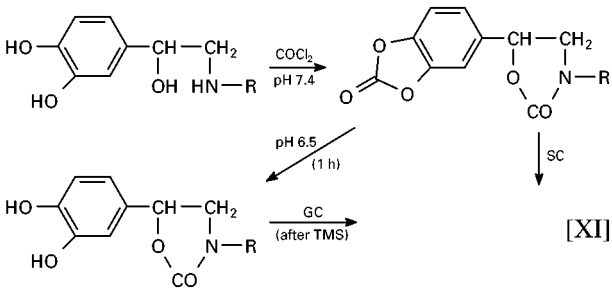


[X]

thyroxine T_4, with a molecular mass of over 1000 Da, is one of the largest compounds ever analysed by GC, the haloalkyl moiety providing relatively high volatility and high electron-capture response. However, the method came at a time when immunoassay was developed as an alternative and further applications of this derivatization method have not been pursued.

The potential of DCTFA to act as a reagent for detector-oriented derivatization has not been fully explored. As shown in the scheme in Figure 4, application to various classes of carboxylic acids still remain to be exploited.

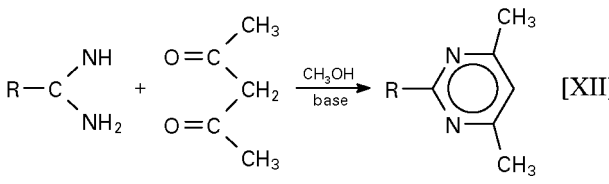
Phosgene, dissolved in toluene or liberated from trichloromethyl (TCM) chloroformate or bis(TMC) carbonate, is able to cyclize adjacent groups of amino alcohols, aromatic diols, etc., as shown for catecholamines. The cyclic carbonate is not sufficiently stable and must be transformed to, for example, a TMS ester (reaction [XI]). Phosgene-induced cyclization to oxazolidones has been used for the GC analysis of enantiomers of pharmaceutically important amino alcohols. More details of the use of this reagent can be found in the review by Gyllenhaal and Vessman (see Further Reading).



[XI]

Pyrimidines

Of the selective procedures for bifunctional compounds that result in cyclized products, the last to be described is that in which pyrimidines are formed by the action of acetylacetone or hexafluoroacetylacetone, or even malonaldehyde, on the guanidino group (reaction [XII]). This could represent a solution for the pre-treatment of arginine but the procedure is time-consuming and requires heating. For further information, see the review by Poole and Zlatkis listed in the Further Reading section.



[XII]

Peralkylation

In addition to amino acid mixtures, another difficult class of compounds to deal with is the acidic metabolites of physiological fluids. Even when compounds with amino groups are not present (except in the case of glycine conjugates), the various classes of acids present – mono-, di- and tricarboxylic acids, keto and hydroxycarboxylic acids, aromatic acids with substituted chains, etc. – make sample preparation particularly challenging. There are one-step, one-reagent procedures that are able to derivatize most protic groups in such a mixture of analytes. These can be effective with alkylating or silylating reagents and also with chloroformates, under the active participation of a component in the reaction medium.

Alkylating Agents

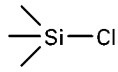
Isopropyl bromide has proved to be a useful reagent for amino acids dissolved in dimethylsulfoxide/sodium hydride, except for the determination of arginine. Methylation of acids with diazomethane has also been used for metabolic profiling despite the formation of artefacts. Resin-mediated methylation of polyfunctional acids found in fruit juices has also proved successful. Fumaric, succinic, malic, tartaric, isocitric and citric acids, isolated from fruit juices by trapping onto anionic ion exchange resins, can be efficiently converted to methyl esters by reaction with methyl iodide in both supercritical carbon dioxide and acetonitrile. To provide for the analysis of even short chain fatty acids in serum, a procedure has been developed with benzyl bromide. This has been successfully employed for serum and urine organic acid profiling. The method cannot be used for citric acid or sugar-related acids.

Silylating Agents

Silylation is the most widely used method for metabolic profiling, especially for urinary organic acids. On the other hand its use for amino acids has a number of disadvantages as already mentioned. For some applications, silylating agents are too powerful in that they are able to react with compounds which will not elute from the column. The most popular reagents are listed in **Table 3** and many others are described in detail in the Fluka handbook edited by van Look (see Further Reading). For metabolic profiling the TMS donors are used much more frequently than the TBDMS donors although the latter are more convenient for treating amino groups in those applications where greater hydrolytic stability is required. Reactivity of functional groups towards silylation is as follows: alcohols > phenols > acids > amines > amides. Disadvantages include the need to operate under

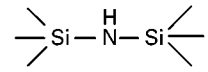
Table 3 Reagents for silylation: (I) most common TMS donors; (II) a TBDMS donor; and (III, IV) reagents for special application

(I) *Trimethylchlorosilane (TMCS)*



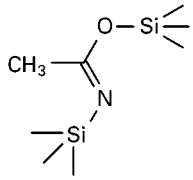
First silylation reagent prepared (1944)
Rarely used in analytical applications alone, rather in mixtures with HMDS and pyridine

Hexamethyldisilazane (HMDS)



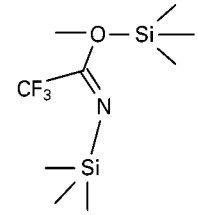
One of the earliest (1957)
Favorable solvating properties for many compounds
Not a strong silyl donor but more selective

N,O-Bis(TMS)acetamide (BSA)



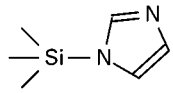
First reported in 1963 as a more potent TMS donor; usually in combination with TMCS (1–20%)

N,O-Bis(TMS)trifluoroacetamide (BSTFA)



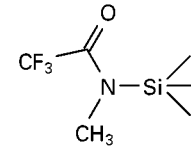
Most widely used for silylation in general (prepared 1968)
Very versatile, reacting with all the common protic sites in organic material present; more volatile by-products!

TMSimidazole (TMSIM)



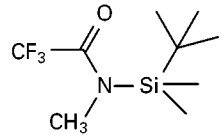
Prepared in 1965; considered to be the strongest reagent available for silylation of hydroxyl groups

N-Methyl-N-TMS-trifluoroacetamide (MSTFA)



Introduced in 1969, has become one of the most important reagents; can be used for all protic groups and its by-products are more volatile than BSTFA

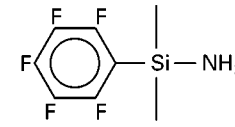
(II) *N-Methyl-N-t-butyldimethylsilyl-trifluoroacetamide (MTBSTFA)*



Alternative silyl derivatives for enhanced hydrolytic stability (1975/1980) increasingly popular, suitable even for amino acids

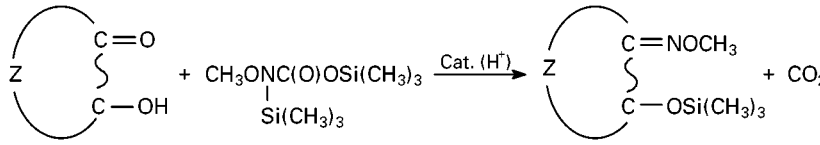
(III) *Pentafluorophenyldimethylsilyl (flophemesyl)* reagents

halogenated reagents for EC detection



(1975), particularly suitable for analysis of sterols; silylation power: flophemesylamine > flophemesylchloride

(IV) *N-Methyoxy-N,O-bistrimethylsilyl carbamate (BSMOC)*
for simultaneous oximation & silylation at room temperature (reported 1986) as follows:



anhydrous conditions, to heat the sample and to inject a reactive mixture onto the column. This means that the compounds of interest have to be isolated, the extraction medium evaporated and the column replaced more frequently. For further information on profiling see the review by Sweetman.

Chloroformates

Chloroformates are known in organic chemistry for their ease of coupling to acids, resulting in the formation of so-called mixed anhydrides:

$$\text{X-COOH} + \text{Cl-COOR} \rightarrow \text{X-COOCOO-R} + \text{HCl}$$

which are, in most cases, stable enough for GC analysis and potentially even reactive enough with amino acids to give peptides. Coupling with an acid is mostly successful in an organic solvent and in the presence of a strong base such as triethylamine (TEA). Considerable effort has been expended into finding reaction conditions to accelerate decarboxylation of the

mixed anhydride to the ester but with the exception of 2-keto acids the process was far from smooth. However, it was found fortuitously that on a microscale the presence of pyridine in a mixture with acetonitrile or water and alcohol results in immediate esterification. The alkyl chloroformates previously used for treating amines and phenols only suddenly became the general-purpose reagent.

The reaction mechanism is based on alcoholysis of the intermediate alkoxycarbonyl ester (the mixed anhydride). On the numerous applications published since 1990, the results for amino acids are especially impressive. It has been found that the alkyl group of the reagent and the alcohol need not be the same and different combinations lead to a variety of esters. In the field of metabolic profiling there is a report on simultaneous analysis of amino acids with other classes of compounds in serum without the need to isolate the analytes from the matrix. Profiling of urinary organic acids, for example, has been made possible after a simple sample pretreatment. Details are given in the review by Hušek (see Further Reading).

Conclusion

Silylating reagents were introduced in the 1960s and have been widely accepted as general purpose derivatizing reagents for GC, especially for polyfunctional compounds where derivatization is reduced to a one-step process. In the 1990s chloroformates were discovered as another family of powerful reagents which, in conjunction with a component of the medium, readily enable derivatization of many hydrogen-containing groups. They bring the additional advantage of derivatization in aqueous media, which often considerably simplifies the sample work-up. Last but not least, advanced alkylation/esterification procedures allow the simultaneous derivatization and extraction of analytes in sample matrices. The present emphasis and future advances will focus on simplification and speed-up of sample preparation methods by process automation and combination of derivatization with work-up procedures.

See also: **II/Chromatography: Gas:** Detectors: Mass Spectrometry; Detectors: Selective. **III/Acids:** Gas Chromatography. **Amino Acids:** Gas Chromatography.

Further Reading

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Detectors: General (Flame Ionization Detectors and Thermal Conductivity Detectors)

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The two most common detectors for use after separation by gas chromatography (GC) are the flame ionization detector (FID) and the thermal conductivity detector (TCD). They are considered general (nonselective) detectors since they respond to virtually all components that they encounter. In a strict sense of course this is not true, especially for the FID which does not respond to fixed gases or to gases commonly used as carrier gases. None the less, it is responsive to most components of interest and is clearly not selective in comparison to an electron-capture or nitrogen–phosphorus detector.

It is sometimes useful to denote detectors as ionizing or nonionizing depending on their mode of