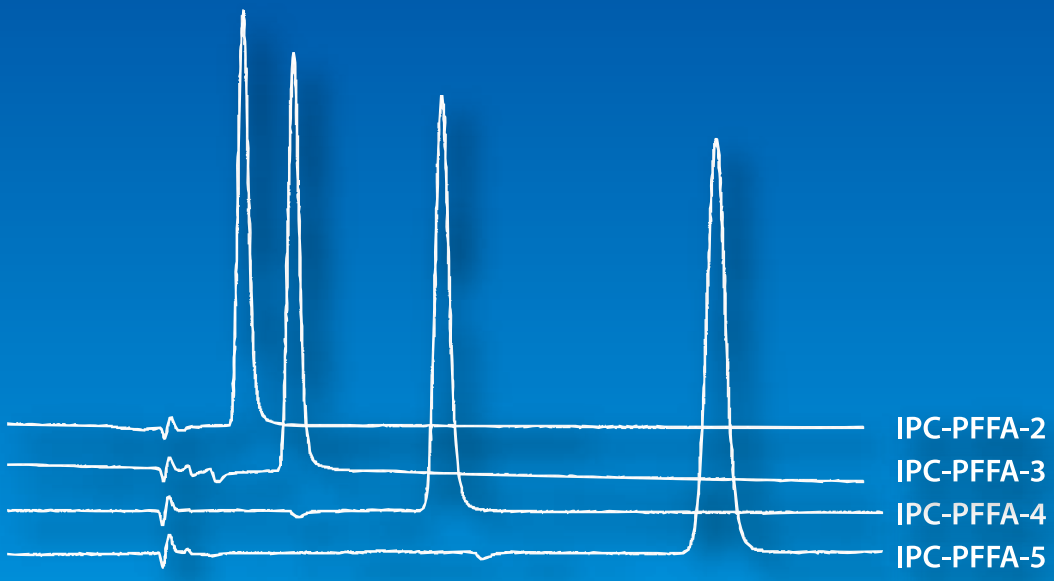
Ion-Pair Reagents for HPLC



Ion-Pair Chromatography for Acidic Samples

Ion-Pair Chromatography for Basic Samples

Ion-Pair Reagents for HPLC

Ion-exchange chromatography systems have previously been utilized in HPLC analysis of ionic samples. Recently, reversed phase partition chromatography using ion-pair reagents has been developed and utilized. The ionic samples form an ion-pair with ion-pair reagents in the mobile phase to become electrically neutral. The increase in hydrophobic character of the ion-pair results in a greater affinity for the reverse stationary phase and leads to sample resolution.

Acidic Samples : $R{-}COO^- + R'_4N^+ \longrightarrow R{-}COO^-\ {}^+NR'_4$

Basic Samples : $R{-}\overset{+}{N}H_3 + R'{-}SO_3^- \longrightarrow R{-}\overset{+}{N}H_3\ {}^-O_3S{-}R'$

UV and fluorescence detectors are widely used. Therefore ion-pair reagents must lack UV absorption and fluorescence themselves to obtain highly sensitive detection of samples. The UV absorption of sodium alkanesulfonates and quaternary ammonium salts is minimal so that these reagents can be used for reliable HPLC analysis. On the other hand, when a sample lacks sufficient UV absorption or fluorescence, the use of sodium 9,10-dimethoxyanthracene-2-sulfonate allows for high-sensitivity detection as a fluorimetric ion-pair reagent.

Recently, use of LC-MS in which mass spectrometry is incorporated in HPLC as a detector has become widespread. Sodium alkanesulfonates, a general ion-pair reagents, being non-volatile crystals pose a problem in that they contaminate the interface. The IPC-PFFA series is made of highly volatile ion-pair reagents allowing for continuous LC-MS analysis without contaminating the interfaces.

Ion-Pair Chromatography for Acidic Samples

- Analysis is performed with pH adjusted to 7.5 with the addition of quaternary ammonium salts to the mobile phase.
- Acidic samples form an electrically neutral ion-pair with the quaternary ammonium salt and are retained in the reverse phase systems.
- The ion-pair reagents for acidic samples for LC-MS are supplied as 0.5 M aqueous solutions and were adjusted to pH 7.5. The solution can be used as a neutral mobile phase after dilution with the LC solvents (acetonitrile/water or methanol/water) to 5 mM. Since the acidic substances are ionized under the neutral conditions, they are facilitated to form an ion-pair.

[Examples]

1. When 0.5 mol/L Tetrabutylammonium Phosphate is used:
   The reagent (10 mL) is diluted to 1 L with an aqueous solvent such as methanol - water.
   (pH adjustment is not required because the reagent is already buffered.)

2. When Tetrabutylammonium Hydroxide is used:
   1) The reagent (12.5 mL) is diluted to 1 L with an aqueous solvent such as methanol - water.
   2) The pH is adjusted to 7.5 by the addition of an aqueous phosphoric acid (10%).

$R_4{-}\overset{+}{N}\ X^-$

I0363	IPC-TEA-OH	(Tetraethylammonium Hydroxide) (10% in Water)			25mL
I0364	IPC-TBA-OH	(Tetrabutylammonium Hydroxide) (10% in Water)		25mL	100mL
I0365	IPC-TBA-Br	(Tetrabutylammonium Bromide)	25g	100g	500g
I0366	IPC-TBA-Cl	(Tetrabutylammonium Chloride)	5g	25g	
I0367	IPC-TBA-P	(Tetrabutylammonium Phosphate) (0.5mol/L in Water)		10mL	100mL
I0368	IPC-TBA-HS	(Tetrabutylammonium Hydrogen Sulfate)	25g	100g	
I0453	IPC-DTMA-Cl	(Dodecyltrimethylammonium Chloride)	25g	500g	

for LC-MS

$R_2{-}\overset{+}{N}H_2\ X^-$

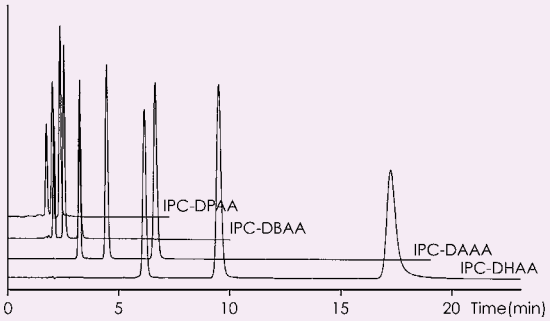
A5703	IPC-DPAA	(Dipropylammonium Acetate) (*ca.* 0.5mol/L in Water)			10mL
A5702	IPC-DBAA	(Dibutylammonium Acetate) (*ca.* 0.5mol/L in Water)		10mL	100mL
A5704	IPC-DAAA	(Diamylammonium Acetate) (*ca.* 0.5mol/L in Water)		10mL	100mL
A5705	IPC-DHAA	(Dihexylammonium Acetate) (*ca.* 0.5mol/L in Water)		10mL	100mL

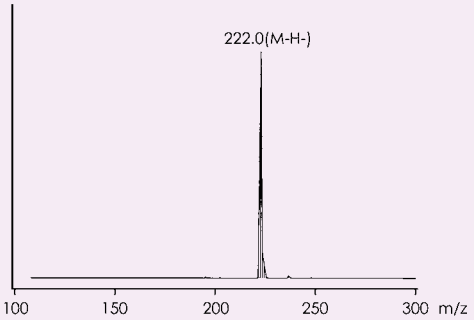
Using of IPC-DRAA **Aminonaphthalenesulfonic Acid**

Column : Kaseisorb LC ODS 2000 2.0 mm I.D. X 150 mm
Mobile phase : CH_3CN / H_2O = 30 / 70 containing 5mM IPC Reagent (Dialkylammonium Acetate)
Flow rate : 0.2 mL/min
Temperature : 25˚C

Detection : UV 254 nm
MS : 1100 MSD (Agilent) ESI (Negative)

Sample : 1. 4-Amino-1-naphthalenesulfonic Acid
2. 1-Amino-8-naphthalenesulfonic Acid
3. 8-Amino-2-naphthalenesulfonic Acid





Ion-Pair Chromatography for Basic Samples

- Analysis is performed by the addition of sodium alkanesulfonate to the mobile phase.
  The basic samples form an electrically neutral ion-pair with sodium alkanesulfonate and are retained in the reverse phase system.
- In the case of sodium alkanesulfonate, the greater the number of carbons in the alkyl group, the greater the partition ratio.
- The solubility of the products such as sodium 1-decanesulfonate (IPC-ALKS-10) may decrease depending upon the composition of the mobile phase solvents; especially after the addition of the buffer for pH adjustment. Resulted turbidity of the mobile phase and crystal formation may interfere with the analysis. To avoid the trouble, modification of the solvent system composition should be considered.
- The ion-pair reagents for basic samples in LC-MS analysis are supplied as 0.5 M aqueous solutions. The solution can be used as an acidic mobile phase after dilution with the LC solvents (acetonitrile/water or methanol/water) to 5 mM. Since the basic substances are ionized under the acidic conditions, they are facilitated to form an ion-pair.
- We launched the high-quality products of PFFA-6, 7 and 8 (A5722, A5721, A5720) for high-sensitive detections.

[Examples]
1) Sodium 1-Heptanesulfonate 1.011 g (0.005 mol) is weighed out.
2) The reagent is dissolved in 1 L of an aqueous solvent such as methanol - water.
3) The pH is adjusted to 3.5 by the addition of aqueous phosphoric acid (50%).

$R—SO_3^-\ Na^+$

I0341	IPC-ALKS-3	(Sodium 1-Propanesulfonate)			5g 25g
I0342	IPC-ALKS-4	(Sodium 1-Butanesulfonate)			5g 25g
I0343	IPC-ALKS-5	(Sodium 1-Pentanesulfonate)	5g	25g	100g
I0344	IPC-ALKS-6	(Sodium 1-Hexanesulfonate)	5g	25g	100g
I0345	IPC-ALKS-7	(Sodium 1-Heptanesulfonate)	5g	25g	100g
I0346	IPC-ALKS-8	(Sodium 1-Octanesulfonate)	5g	25g	100g
I0347	IPC-ALKS-9	(Sodium 1-Nonanesulfonate)			5g 25g
I0348	IPC-ALKS-10	(Sodium 1-Decanesulfonate)			5g 25g
I0349	IPC-ALKS-11	(Sodium 1-Undecanesulfonate)			5g 25g
I0350	IPC-ALKS-12	(Sodium 1-Dodecanesulfonate)			5g 25g
I0351	IPC-ALKS-13	(Sodium 1-Tridecanesulfonate)			5g 25g
I0352	IPC-SDS	(Sodium Dodecyl Sulfate)	5g	100g	500g

for LC-MS

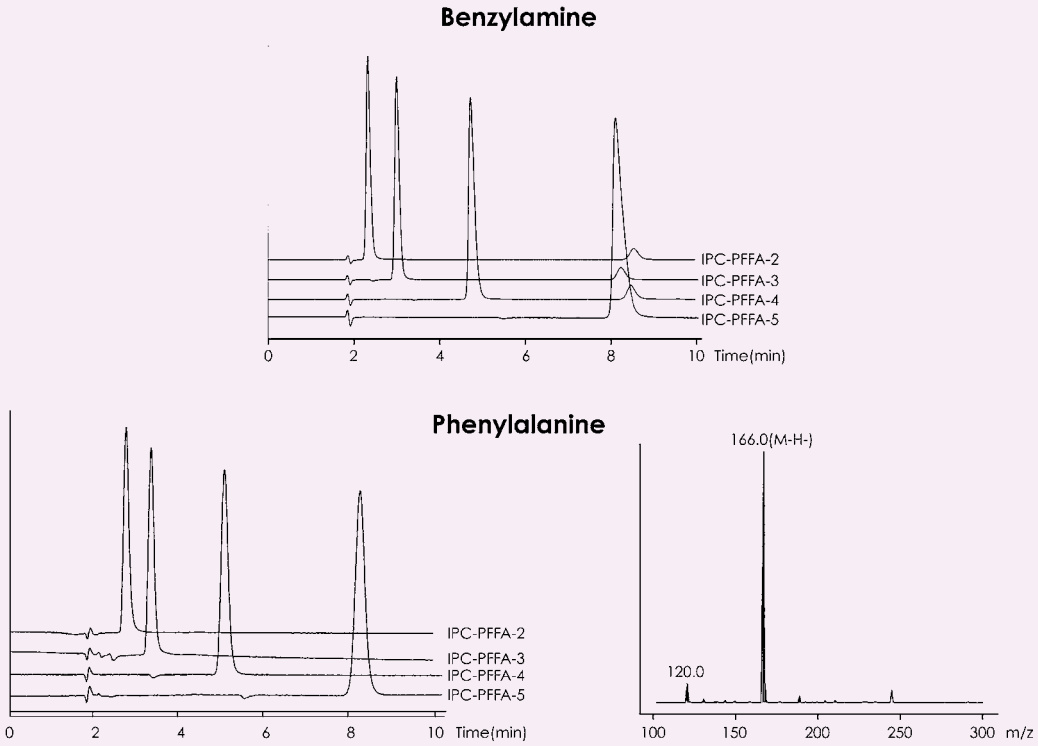
$Rf—COOH$

A5711	IPC-PFFA-2	(Trifluoroacetic Acid) (*ca.* 0.5mol/L in Water)			10mL
A5712	IPC-PFFA-3	(Pentafluoropropionic Acid) (*ca.* 0.5mol/L in Water)			10mL
A5713	IPC-PFFA-4	(Heptafluorobutyric Acid) (*ca.* 0.5mol/L in Water)		10mL	100mL
A5714	IPC-PFFA-5	(Nonafluorovaleric Acid) (*ca.* 0.5mol/L in Water)			10mL
A5715	IPC-PFFA-6	(Undecafluorohexanoic Acid) (*ca.* 5mmol)			1sample

A5716	IPC-PFFA-7	(Tridecafluoroheptanoic Acid) (*ca.* 5mmol)	1sample	
A5717	IPC-PFFA-8	(Pentadecafluorooctanoic Acid) (*ca.* 5mmol)	1sample	
A5722	IPC-PFFA-6 HG	(Undecafluorohexanoic Acid High Grade)	1g	5g
A5721	IPC-PFFA-7 HG	(Tridecafluoroheptanoic Acid High Grade)	1g	5g
A5720	IPC-PFFA-8 HG	(Pentadecafluorooctanoic Acid High Grade)	1g	5g

Using of IPC-PFFA

Column : Kaseisorb LC ODS 2000 2.0 mm I.D. X 150 mm
Mobile phase : CH_3OH / H_2O = 40 / 60 containing 5 mM IPC Reagent
Flow rate : 0.2 mL/min
Temperature : 25°C
Detection : UV 254 nm
MS : 1100 MSD (Agilent) ESI (Positive)



Fluorimetric Ion-Pair Reagent

A5701 Sodium 9,10-Dimethoxyanthracene-2-sulfonate 1g

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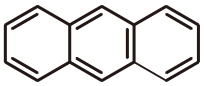
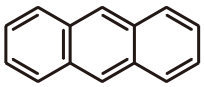
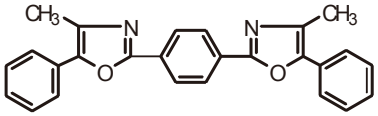


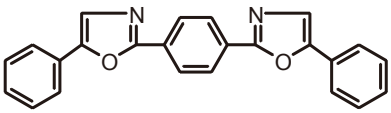
1 sample
[I0405]



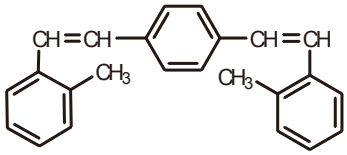
25g / 100g / 500g
[A0495]



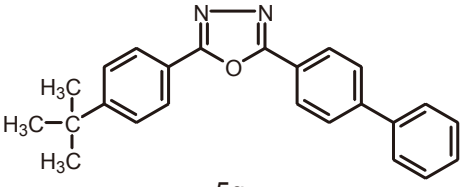
5g / 25g
[B0499]



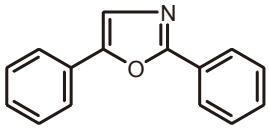
1g / 5g / 25g
[B0509]



5g / 25g
[B1024]



5g
[B1767]



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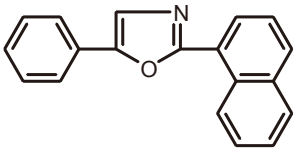
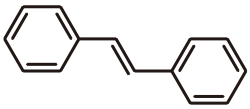
[D0902]



1g
[N0068]



25g / 100g / 500g
[S0090]

25g / 100g / 500g
[T0020]

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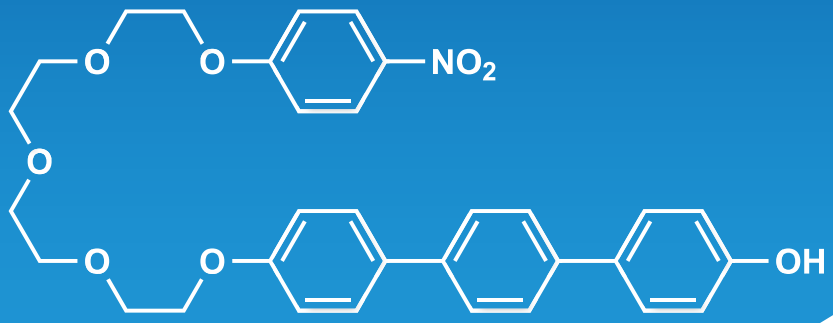
用于微量铯可视化的荧光探针

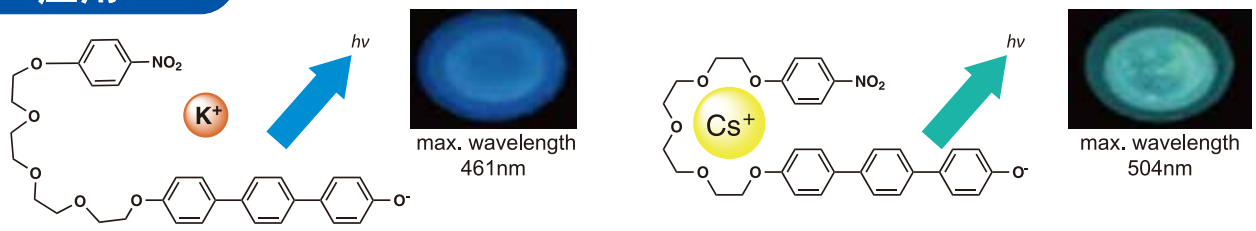


[C2806]

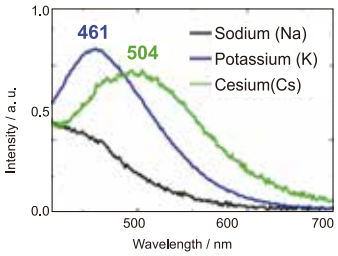
优势

- 土壤中含有的粒子状铯离子可通过绿色荧光检出
- 植物的茎截面含有的粒子状铯离子可实现可视化

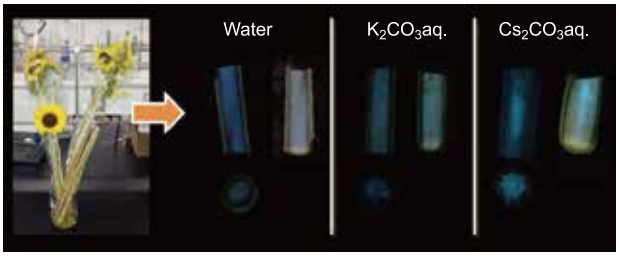
应用



The complex structures and fluorescence properties of C2806 with K^+ or Cs^+
(UV irradiation (365 nm) after addition of a drop of methanol)



Fluorescence spectra of a mixture of C2806 with alkali metals.
(Numbers indicate the wavelength of the fluorescence maximum.)



The photographs show distribution of K^+ and Cs^+ in freeze-dried sunflower stem cross sections under UV irradiation (365 nm).
(image on the left: spraying only with methanol, images on the right: spraying with C2806 in methanol)

Images and data courtesy of the National Institute for Material Science

C2806 Cesium Green 50mg

该产品在Katsuhiko Ariga博士的指导下实现了商品化。

T. Mori, M. Akamatsu, K. Okamoto, M. Sumita, Y. Tateyama, H. Sakai, J. P Hill, M. Abe, K. Ariga, *Sci Technol. Adv. Mater.* **2013**, *14*, 015002.
Patent pending from National Institute for Material Science

使用C2806进行铯粒子可视化检测的方法。

1. 固态下铯离子的可视化

配制0.02wt%的**C2806**的甲醇溶液。
将该溶液滴加到Cs_2CO_3粒子上。
在紫外光下(365nm)可观测到粒子上发射出绿色的荧光。

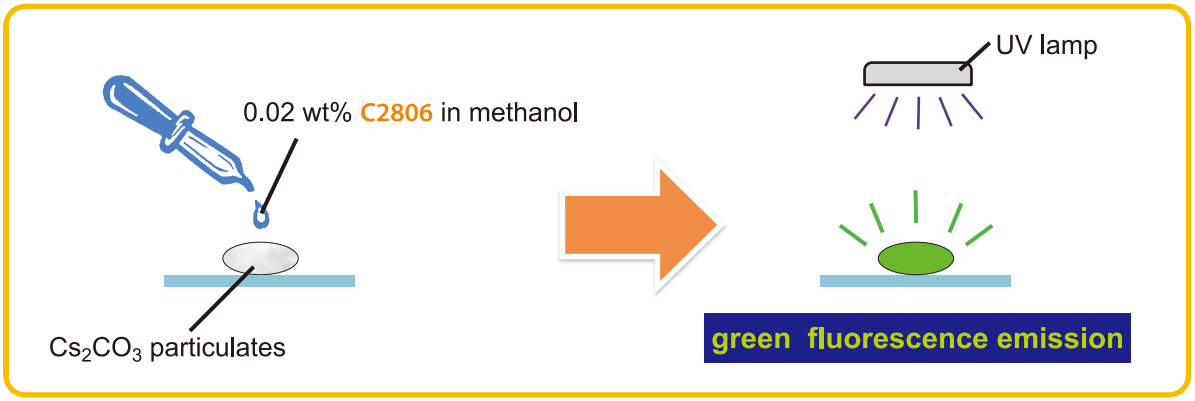


Fig.1 Visualization of cesium ion in a solid state

2. 植物中铯离子的可视化

将向日葵的茎浸入Cs_2CO_3 (1 wt%)的水溶液中几天以吸收铯离子。经冷冻干燥，截面处喷洒**C2806**的甲醇溶液。在紫外光下(365nm)仅吸收铯离子的茎部可观测到绿色的荧光。

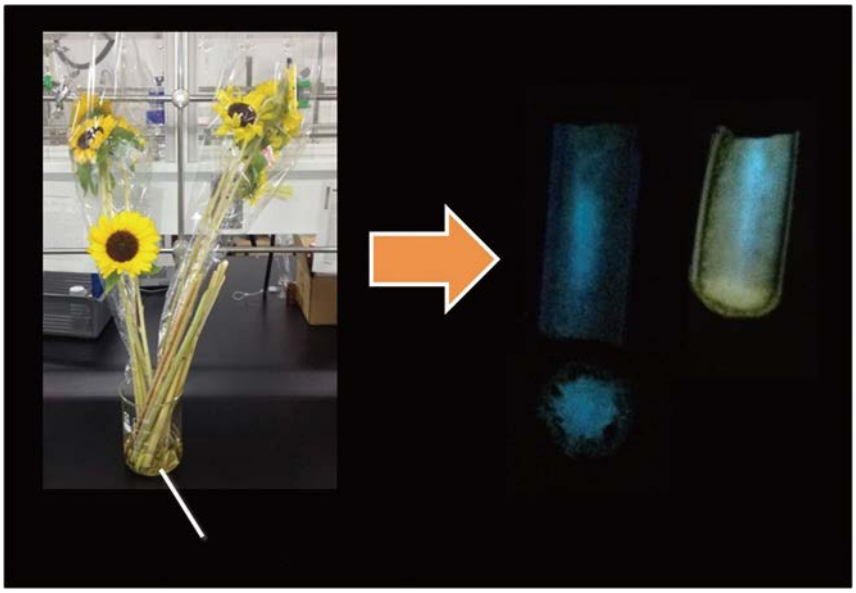


Fig.2 Visualization of cesium ion in plants

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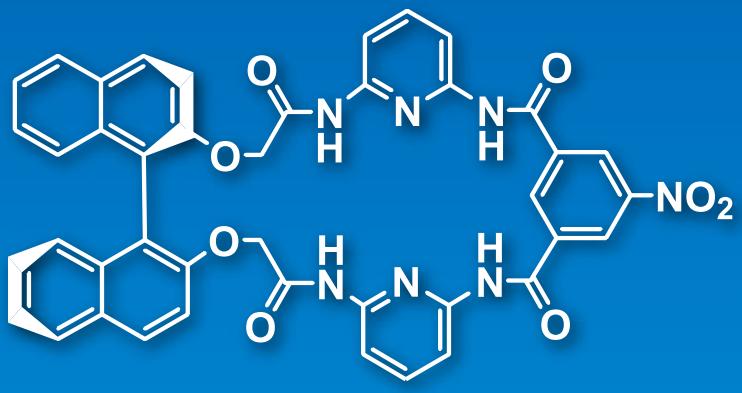
New

用于检测对映体过量的手性位移试剂



[C2184]

优点

- 由于不含有导致信号变宽的顺磁性金属，因此C2184既适用于高位、也适用于低位NMR光谱仪。
- 多种化合物的对映体纯度可被检测出。
- 把C2184加入到$CDCl_3$溶解的目标样品核磁管中，就能得到显示化学位移不对等的NMR波谱。

应用

Chart 1.

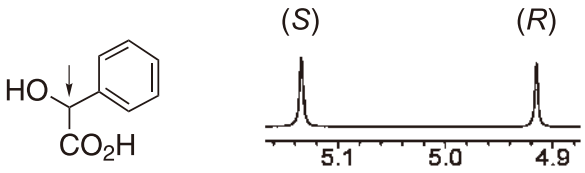
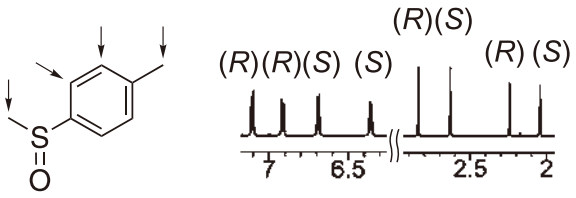


Chart 2.



Chirabite-AR (7mg) in $CDCl_3$ at 22°C

T. Ema, D. Tanida, T. Sakai, *Org. Lett.* **2006**, *8*, 3773.
T. Ema, D. Tanida, T. Sakai, *J. Am. Chem. Soc.* **2007**, *129*, 10591.

C2184 Chirabite-AR 100mg

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ANALYTICAL

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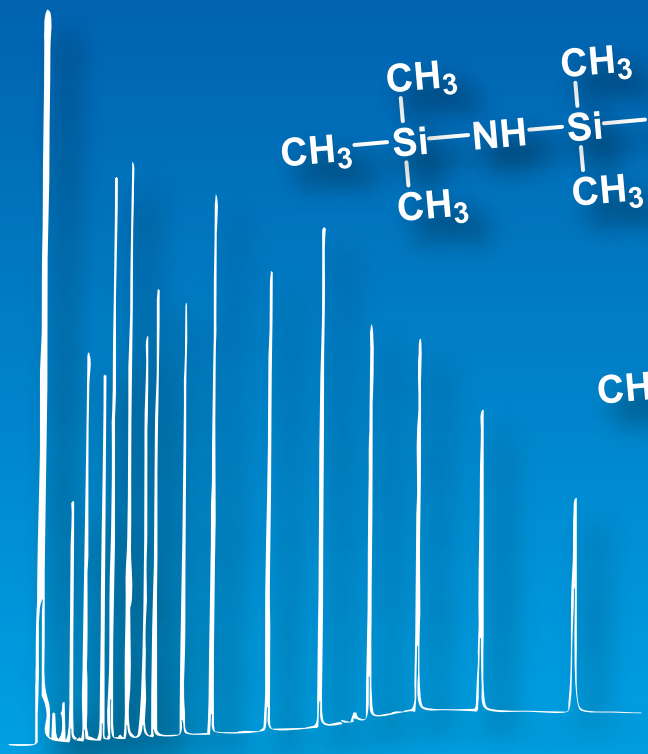
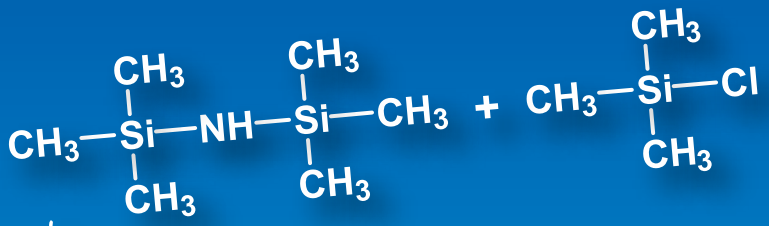
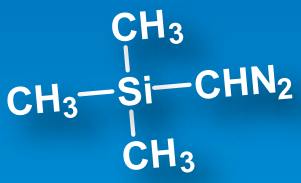
GC Derivatization Reagents







Trimethylsilylation

Acylation

Silylation

Esterification

Other Pretreatment

CONTENTS

■Esterification

■Other Pretreatment

Trimethylsilylation

Trimethylsilylating Reagents (TCI-Ace)

Gas Chromatography (GC) is widely used for analysis of various kinds of samples. The range of analytes has continued to expand to trace components in biological and environmental fields. As a result, GC derivatizing reagents for specific purposes have been under increasing demand.

TCI-Ace trimethylsilylating reagents are GC derivatizing reagents quality-controlled for analyzing trace-level components. These reagents are highly purified so that impurities with high boiling point that would disturb the analysis (the component whose retention index is over 1500) are kept below 20ppm per component.

1. Products TCI-Ace Trimethylsilylating Reagents

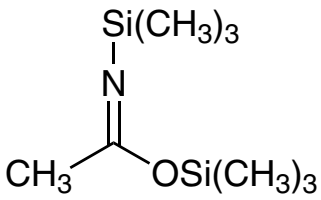
Code	Item	Volume	Vessel
A5601	BSA [=*N,O*-Bis(trimethylsilyl)acetamide]	5 mL	Vial
A5602	TMS-BA (BSA 25% in Acetonitrile)	5 mL	Vial
A5603	BSTFA [=*N,O*-Bis(trimethylsilyl)trifluoroacetamide]	5 mL	Vial
A5604	TMS-HT (=HMDS and TMCS in Anhydrous Pyridine)	5 mL	Vial
A5605	TMS-Imidazole (=SIM, *N*-Trimethylsilylimidazole)	5 mL	Vial

2. Precautions for Safe Handling

* Avoid moisture and keep container tightly sealed. Store in an explosion-proof refrigerator.
* Do not breathe dust/fume/gas/mist/vapors/spray.
* Avoid contact with the skin, eyes, mouth and mucous membranes.
* Use a dry syringe or micro-syringe to withdraw reagent from the vial.
* The packing of the vial is made from Teflon-coated rubber. Direct contact with rubber may cause contamination of the reagent by piercing with a needle. The reagent should be used as soon as possible after piercing.

3. Product Details

3.1 A5601 BSA [=*N,O*-Bis(trimethylsilyl)acetamide] 5 mL



[Application]

BSA is highly reactive towards nitrogenous compounds such as amino acids and amides, as well as compounds bearing hydroxyl or carboxyl groups. BSA cannot be used alone for the trimethylsilylation of sugars but can be used with catalytic amounts of chlorotrimethylsilane (TMCS).

BSA is applicable to amino acids, amides, ureas, phenols, carboxylic acids, enols, sulfonic acids, steroids, uric acids, nucleic acids, and sugars.

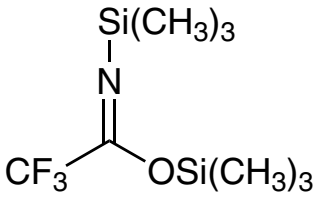
3.2 A5602 TMS-BA (25% BSA in Acetonitrile) 5 mL

TMS-BA might be separated into two layers during cool weather or by storing in a refrigerator. In that case, heat and shake to homogenize before use.

[Application]

Equivalent to BSA.

3.3 A5603 BSTFA [=*N*,*O*-Bis(trimethylsilyl)trifluoroacetamide] 5 mL



[Application]

Equivalent to BSA. BSTFA is effective for Flame Ionization Detector (FID) applications, and excels in activity, volatility, and solubility as compared with BSA. BSTFA by-products have high volatility and minimally disturb the analysis on GC compared to BSA. It is particularly suitable for trimethylsilylation of amino acids. (e.g. alanine and valine need to be heated at 125 °C for 15min.)

3.4 A5604 TMS-HT (=HMDS and TMCS in Anhydrous Pyridine) 5 mL

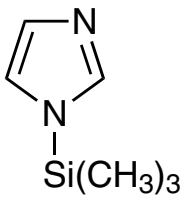
TMS-HT is a pyridine solution that is mainly composed of hexamethyldisilazane and trimethylchlorosilane. Although it sometimes precipitates ammonium chloride crystals during storage, its supernatant can be used.

Hexamethyldisilazane (=HMDS)
Chlorotrimethylsilane (=TMCS)
Pyridine

[Application]

Suitable for hydroxyl groups (e.g. alcohols, sugars, and steroids)

3.5 A5605 TMS-Imidazole (=SIM, *N*-Trimethylsilylimidazole) 5 mL



[Application]

Reacts selectively with hydroxyl groups (e.g. alcohols, sugars, steroids, and uric acids)

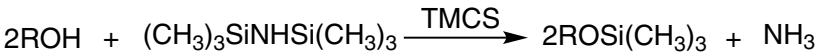
4. Overview

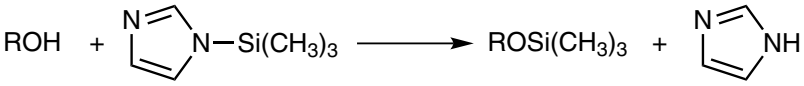
Trimethylsilylating reagents have applicability in wide range of applications such as GC analyses (e.g. separation of structurally similar materials and clinical inspection like analysis of serum amino acids, steroids, uric acids, etc.), protection of reactive groups during peptide/nucleoside synthesis, and for the separation/purification of organic compounds and inorganic acids (boronic acids, arsenic acids, and phosphoric acids, etc.).

Trimethylsilylating reagents are commonly used for GC analysis of compounds having slightly volatile polar functional groups such as hydroxyl groups, carboxyl groups, thiol groups, amino groups and imino groups. TMS reagents can convert these compounds (e.g. sugars, alcohols, phenol, steroids, amino acids, peptide and nucleic acids) into TMS ether, TMS ester, TMS thioether, and *N*-TMS which are thermally stable and volatile. Even an analyte is not stable enough to perform normal pretreatment (e.g. uronic acid) or difficult to trimethylsilylate directly (e.g. sulfonate salts), TMS reagents can be used by preparing appropriate derivatives beforehand (such as sugars, alcohols, and thiols mentioned in the example).

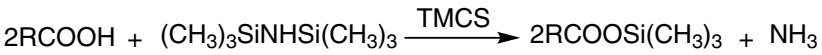
5. Reaction Formula of Trimethylsilylation

Hydroxyl compounds

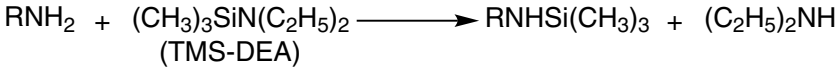




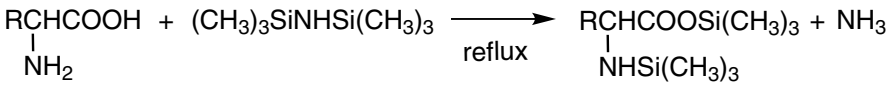
Carboxyl compounds

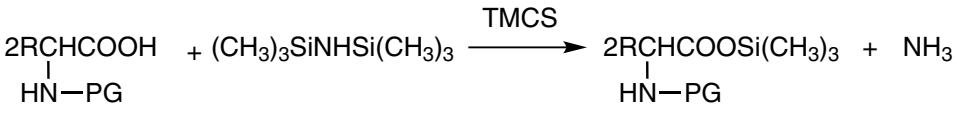


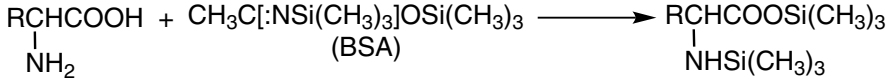
Amino compounds

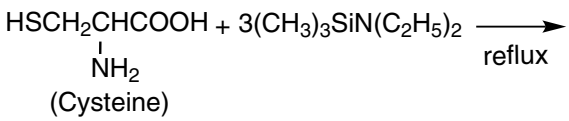


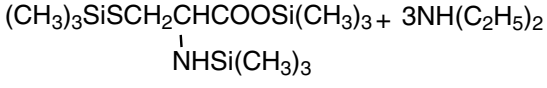
Amino Acids











6. Applications

6.1 General Procedure

[1] Sugars, Alcohols, Steroids, and others

Approx. 1 mg of a sample and either 1 mL of TMS-HT or 1 mL of SIM are placed in a dry vial, and then the vial is tightly sealed and allowed to react by shaking or heating. The supernatant can be used as a GC sample when using TMS-HT because crystals of ammonium chloride will be formed.

[2] Alcohols, Amino Acids, Amines, and others

Approx. 1 mg of a sample and 1 mL of TMS-BA are placed in a dry vial, and the vial is tightly sealed and allowed to react by shaking or heating.

6.2. Practical Application

6.2.1 [Sugars]

[1] Sugars in General

1 mL of TMS-HT is added to 10 mg of sugars. After shaking for 30 sec, the mixture is left for 5 min at room temperature. The supernatant can be used as a GC sample.[1)]

[2] Dissaccharides in Blood and Urine [2)]

A dry sample from 1 mL of blood/urine is added into either 50 µL of BSA :TMCS :pyridine (1:1:2) or 200 µL of BSA : TMCS : pyridine (1:1:5), and then the resulting mixture is allowed to react for 45 min at room temperature or for 20 min at 60 °C.

6.2.2 [Amino acids] [3)]

BSA is added to free amino acids or hydrochloride (5-10 mg), and allowed to react by heating for 1-2 h at 80 °C or for 0.5-1 h at 90 °C. Mainly Bis-TMS adduct is obtained from the free amino acids, whereas tris-TMS adduct is obtained from their hydrochlorides, respectively.

6.2.3 [Catecholamines] [4)]

1mg of norepinephrine is dissolved in 0.1 mL of acetonitrile, and then 0.2 mL of BSA, 0.1 mL of TMCS, and 2 µL of water are added. *N*,*N*,*O*',*O*'',*O*'''-pentakis-TMS adduct is obtained by heating for 2 h at 60 °C. However, the reaction takes 5 h to complete without adding water.

6.2.4 [Steroids]

[1] Hydroxysteroids

Non-sterically hindered hydroxyl group can readily be trimethylsilylated by the general procedure. The reactivity between the positions of hydroxyl groups (such as at 3-, 11-, 16-, 17-, and 20- positions) and trimethylsilylating reagents has been much discussed so far.[5-8)] For example, 10% of TMCS is added to BSA, HMDS or SIM for the trimethylsilylation of 11β-OH, whereas 20% of TMCS is added to BSA or SIM for the trimethylsilylation of 17α-OH as a catalyst, respectively. Full trimethylsilylation of cortols has also been reported.[5-7)]

[2] Methoxime-trimethylsilylation of ketosteroids

(1) 0.5 mL of pyridine and 8 mg of methoxylamine hydrochloride are added to 2 mg of steroids, and then the mixture is allowed to react for 3 h at 60 °C or overnight at room temperature. After extracting with benzene or ethyl acetate, the solvent is evaporated by N_2 flow. 0.2 mL of BSA is added to the residue and it is allowed to react for 3-5 h at room temperature. As for steroids having 11β-OH, 0.005-0.1 mL of TMCS is added as a catalyst.[9)]

(2) 50 µL of 10% pyridine solution of methoxylamine is added to 0.1 mg of steroids, and the resulting mixture is allowed to react for 15 min at 60 °C to complete the reaction. Then 50 µL of SIM is added to the mixture and is allowed to react for 2 h at 100 °C. Cortol is also thoroughly trimethylsilylated by the catalytic action of methoxylamine.

[3] Methoxime-trimethylsilylation of steroid hormones (in urine) [10)]

100 µL of pyridine solution of methoxylamine hydrochloride is added to a dried sample prepared from 5 mL of a sample of urine hydrolyzed enzymatically and undergone clean-up treatment. And then the mixture is allowed to react for 15 min at 60 °C for methoximation. After removing pyridine by N_2 flow, 100 µL of BSTFA : TMCS (5:1, v:v) is added, and the mixture is allowed to react for 2 h at 60 °C to complete the trimethylsilylation.

[4] Dexamethasone [11)]

50 µL of pyridine containing 5 mg of methoxylamine hydrochloride is added to 0.1 mg of a sample, and the mixture is allowed to react for 3 h at 60 °C to complete the reaction of a carbonyl group at the 20-position. To this 50 µL of SIM is added and the mixture is allowed to react for 5 h at 100 °C for tris-trimethylsilylation.

[5] Phytoecdysone[12)]

0.5 mg of steroids is dissolved in 20 µL of SIM, and the mixture is allowed to react for 1 h at 100 °C. All hydroxyl groups are trimethylsilylated, but the 6-positioned carbonyl groups is not affected.

7. References

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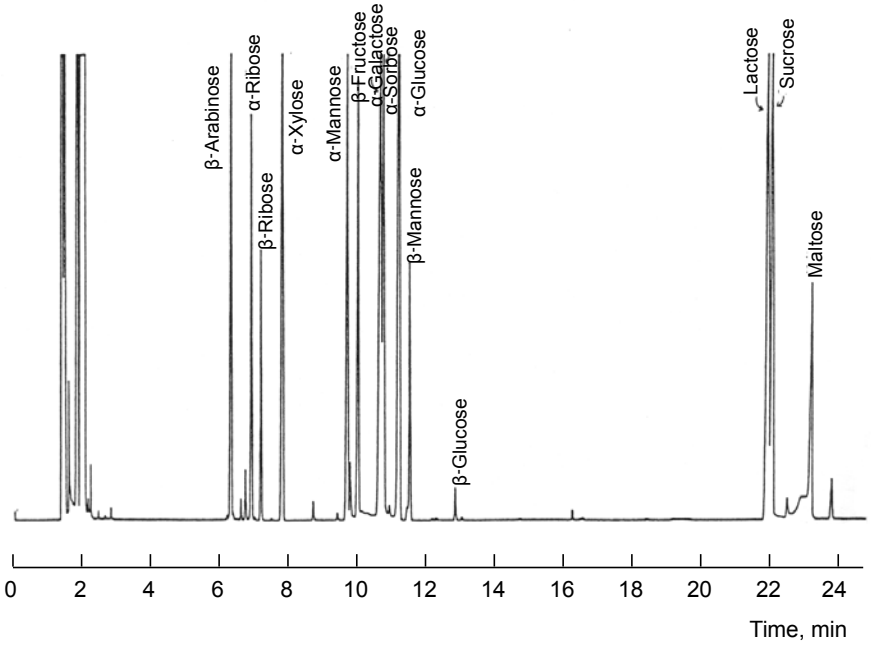
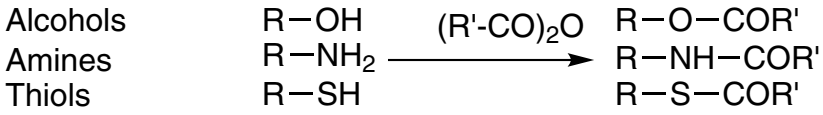
TMS SUGARS

Column : 007-1
25 m × 0.25 mmI.D. × 0.25 µm
Temperature : 150 °C(5 °C / min) ～ 220 °C(10 °C / min) ～ 270 °C
Detector : FID
Inj. Mode : Split
Carrier Gas : He 30 cm/s



Acylation

Acid Anhydrides[1~3)]



T0433	**Trifluoroacetic Anhydride**	**20 mL**	**400 mL**
P0566	**Pentafluoropropionic Anhydride**	**5 g**	**25 g**
H0337	**Heptafluorobutyric Anhydride**		**10 g**

[Application Example]

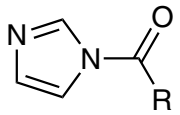
Trifluoroacetylation of alcohols, amines and others[4)]

1-5 mg of sample is dissolved in 0.5 mL of solvent such as acetone or dichloromethane*, and 200 µL of trifluoroacetic anhydride is added. The mixture is allowed to react for 20-30 min at room temperature (or heated to 40 °C if necessary). After removing excess reagent and solvent by N_2-blowing, the residue is dissolved in acetone or other solvents to be used as a GC sample.

*If the sample is difficult to dissolve in such solvents, trifluoroacetic acid can be used as a solvent.

Acylated Imidazoles[5-9)]

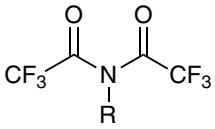
A0694	***N*-Acetylimidazole**	**25 g**	**500 g**
T0670	***N*-Trifluoroacetylimidazole**	**5 g**	**25 g**
H0467	**1-(Heptafluorobutyryl)imidazole**	**5 g**	**25 g**



Acylation reactions can proceed under mild conditions. The resulting imidazoles are inert.

Fluorinated Acetamides[10,11)]

B0986	**Bistrifluoroacetamide**	**5 g**	**25 g**
M0671	***N*-Methylbis(trifluoroacetamide) (=MBTFA)**	**1 mL**	**5 mL**



Trifluoroacetylation of the amino groups, hydroxyl groups and thiols can proceed under mild conditions.

[Application Example]

Trifluoroacetylation of sugars[11)]

5-10 mg sugars are placed in a 2 mL vial, then 0.5 mL of MBTF and 0.5 mL of pyridine are added respectively. The resulting mixture is heated for about 1 h while shaking occasionally. The reaction is completed when the sample is dissolved, which can be used as a GC sample.

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Silylation

Trimethylsilylating Reagents

H0089 1,1,1,3,3,3-Hexamethyldisilazane (=HMDS) 10 mL 100 mL 500 mL
C0306 Chlorotrimethylsilane (=TMCS) 25 mL 500 mL

TMCS is frequently used with HMDS. TMC is easily decomposed by moisture and generates hydrochloric acid gas in the process requiring careful handling.

T0274 TMS-HT 12 mL
T0690 TMS-HT Kit Contents of the Kit: reagent (1mL) vial×8, 2mL empty reaction vial×8

TMS-HT is a pyridine solution whose principal constituents are hexamethyldisilazane (HMDS) and chlorotrimethylsilane (TMCS) and is useful for the trimethylsilylation of hydroxyl and carboxyl groups. If crystals of ammonium chlorid are appeared during storage, the supernatant can be used.

[General Procedure]

1. 1 mL of TMS-HT is added to *ca*. 1 mg of a sample in a dry vessel (preferably a vial of about 2 mL capacity), and then it is sealed and shaken (crystals of ammonium chloride are precipitated). The supernatant is injected into the GC column. In some cases, it is needed to heat to complete the reaction.
2. Apporx. 1 mg of sugar is dissolved in 0.2 mL of pyridine, and 1 mL of TMS-HT is added to the mixture. And then, white precipitate of ammonium chloride appreares immediately. After it is left at room temperature for about 5 min while intermittently shaking (if necessary, the vessel may be heated by directly immersing in a water bath: e.g. for maltose, at 80-90 °C for 2-3 min). The supernatant is used as a GC sample.

Note: If a liquid is poured into the sealed reaction vial in the kit, it is recommended to reduce the pressure of the vial in advance by using a syringe. For a solutions of sugars, use TMS-PZ.

[Applications]

Those that can be trimethylsilylated at room temperature

Alcohols (such as 2-methyl-2-butanol, stearyl alcohol, and oleyl alcohol),[1,2] sugars (such as xylose, cellobiose, and trehalose),[3-11,27] amino sugars, phenols (such as *o*-cresol, *m*-cresol, *p*-cresol, tricresol, and guaiacol),[12] organic acids (such as benzoic acid, salicylic acid, gentisic acid, and gallic acid),[13-17] amino acids (trimethylsilylation of DNP-methyl ester derivatives such as serine, threonine, and hydroxyproline),[18] catecholamine,[19] bile acids (trimethylsilylation of methyl ester derivatives),[20] fatty acids,[1] acids of citric acids cycle (such as α-keto glutaric acid, oxalacetic acid),[21] alkaloids (such as morphine, codeine.),[22] and steroids.[23,28]

Those that can be trimethylsilylated at about 100 °C and for 1 h

Sugar phosphate salts (such as D-erythrose-4-phosphate, D-ribose-5-phosphate, D-fructose-6-phosphate, D-glucose-6-phosphate, and D-glucose-1-phosphate) [24] and nucleoside (such as adenosine, inosine, uridine, deoxyuridine, thymidine, xanthosine, cytidine, and guanosine).[25,26]

Keto acids[21]

In order to obtain positive results in the trimethylsilylation of α-ketoglutaric acids and oxaloacetic acids in GC, their oximes are first prepared, then converted into TMS-oxime derivatives. 10 mg of a sample and 10 mg of hydroxylamine hydrochloride are dissolved in 1 mL of dry pyridine and left for 10 min at room temperature. After that, 1 mL of TMS-HT is added, and the mixture is left for 5 min at room temperature.

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B0511 *N,O*-Bis(trimethylsilyl)acetamide (=BSA) 10 mL 100 mL
B0911 *N,O*-Bis(trimethylsilyl)acetamide Kit (=BSA Kit)
Contents of the Kit: reagent (1 mL) × 8, 2 mL blank vial for reaction×8

[Application]

BSA is highly reactive towards alcohols and carboxylic acids, as well as nitrogenous compounds (such as amino acids,[4,5,7] amides, ureas,[4] phenols, carboxylic acids, enol compounds,[3] sulfonic acids, steroids,[9,10,11] nucleic acids,[2] sugars[1,8]).

[General Procedure]

BSA is added to 10-50 mg of a sample placed into a dry vessel, and the vessel is sealed tightly. If necessary, it is heated at 70-80 °C for 30 min-1 h.

[Applications]

Steroids

0.2 mL of *N*-trimethylsilylimidazole (= SIM) is added to 1-5 mg of a sample in 0.1 mL of pyridine. After sealing the vessel, the mixture is left for 0.5-1 h at room temperature. The mixture usually can be used for a sample for GC. In the case of a sterically hindered alcohols, it is recommended to use BSA and TMCS together with SIM. For ketosteroids, after leaving for 3 h at room temperature, 0.2 mL of BSA is added and left for further 2 h at room temperature. The resulting transparent solution can be used for GC. By this method, the carbonyl group is converted into an enol TMS ether, and these derivatives can bevery useful for GC applications. Furthermore, the reaction improves by adding a trace ammounts of TMCS.

Cortol[11]

Trimethylsilylation of cortol with 3:3:2 volume mixture of SIM, BSA and TMCS affords penta-TMS derivative cortol. If BSA is used alone, hydroxyl groups at 3-, 20-, and 21-position are trimethylsilylated. When BSA and TMCS are used together, hydroxyl groups at 3-, 11-, 20-, and 21-position are trimethylsilylated.

Sulfonic acids and Sulfonate salts

After the conversion to thiol derivatives, BSA is added. The mixture is trimethylsilylated by leaving for 10 min at *ca*. 80 °C.

B0510 *N,O*-Bis(trimethylsilyl)acetamide (25% in Acetonitrile) (=TMS-BA) 12 mL
T0691 *N,O*-Bis(trimethylsilyl)acetamide Kit (25% in Acetonitrile) (=TMS-BA Kit)
Contents of the Kit: reagent (1 mL) × 8, 2 mL blank vial for reaction×8

TMS-BA is an acetonitrile solution of bis(trimethylsilyl) acetamide. It may be separated into two layers in winter or when stored in a cold place. If so, it should be homogenized by heating and shaking before use.

[Application]

Equivalent to BSA's.

[General Procedure]

1. *ca*. 1 mg of sample and 1 mL of TMS-BA is placed into a dry vessel (about 2 mL of vial is preferable). After sealing the vessel, the reaction is proceeded by shaking or heating (e.g. for leucine, threonine, by heating for 15 min at 125 °C) to result a transparent solution. And then it is directly injected into GC.
2. *ca*. 0.5-1 mg of steroid is dissolved in 0.05-0.1 mL of an appropriate solvent (such as pyridine and acetonitrile) and the mixture is poured into 1 mL of TMS-BA. It is left at room temperature or the vial is directly heated with water bath (e.g. for estriol, for 20 min at 78-80 °C), and then it can be used as GC sample.

Note: If a liquid is injected into the sealed reaction vial in the kit, it is recommended to reduce air pressure in the vial in advance by using a syringe.

B0830 *N,O*-Bis(trimethylsilyl)trifluoroacetamide (=BSTFA) 5 mL 25 mL
B0912 *N,O*-Bis(trimethylsilyl)trifluoroacetamide Kit (=BSTFA Kit)
Contents of the Kit: reagent (1mL) vial×8, 2mL empty reaction vial×8

[Application]

Equivalent to BSA. BSTFA is useful in Flame Ionization Detector (FID), and excels in activity, volatility, and solubility as compared to BSA. BSTFA by-products have high volatility and minimally disturb the analysis on GC compared to BSA. It is particularly suitable for trimethylsilylation of amino acids.[4-6,13,14] (e.g. for alanine and valine, they can be trimethylsilylated by heating at 125 °C for 15 min.)

[Application Example]

Amino acids[12]

1 mg of a dry sample is placed into a vial, and then 0.24 mL each of acetonitrile and BSTFA is added. After sealing the vial, it is shaken to become a homogenized solution, and then heated with an oil bath (150 °C) for 15 min. After cooling, it can be used as a GC sample.

References

1) H. Mizunuma, K. Minakawa, *Igaku No Ayumi* **1968**, *65,* 679.
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4) F. Shahrokhi, C. W. Gehrke, *J. Chromatogr.* **1968**, *36*, 31.
5) E. D. Smith, K. L. Shewbart, *J. Chromatogr. Sci.* **1969**, *7*, 704.
6) M. R. Guerin, W. D. Shults, *J. Chromatogr. Sci.* **1969**, *7*, 701.
7) K. A. Caldwell, A. L. Tapple, *J. Chromatogr.* **1968**, *32*, 635.
8) Y. Masada, K. Hashimoto, T. Inoue, T. Sawada, *YAKUGAKU ZASSHI* **1969**, *89,* 734.
9) E. C. Horning, M. G. Horning, N. Ikekawa, E. M. Chambaz, P. I. Jaakonmaki, C. J. W. Brooks, *J. Gas Chromatogr.* **1967**, *5*, 283.
10) E. M. Chambaz, G. Maume, B. Maume, E. C. Horning, *Anal. Lett.* **1968**, *1(12)*, 749.
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14) C. W. Gehrke, K. Leimer, *J. Chromatogr.* **1970**, *53*, 201.
15) K. Bergström, J. Gürtler, R. Blomstrnd, *Anal. Biochem.* **1970**, *34*, 74.

T0590 *N*-Trimethylsilylacetamide (=*N*-TMS-acetamide) 25 g

[Application]

Ascorbic acid (Vitamin C)

50 mg of a sample and 50 mg of octadecane (internal standard) is dissolved in 10 mL of dry pyridine. Next, 1.5 g of *N*-TMS-acetamide is added, and the mixture is left for over 4 h at room temperature.

References

M. Vecchi, K. Kaiser, *J. Chromatogr.* **1967**, *26*, 22.

M0536 *N*-Methyl-*N*-trimethylsilylacetamide (=*N*-Methyl-*N*-TMS-acetamide) 10 g 25 g

[Application]

Amino acid

0.1 mL of *N*-Methyl-*N*-TMS-acetamide is added to a 1-2 mg samplevial and is sealed tightly and stirred for 5 min at room temperature. If the sample does not dissolve, heat to 60-100 °C. The formation of a transparent solution indicates reaction completion. The reaction is then directly injected into GC for analysis.

Others

amines, fatty acids, polyols, sugars, phenols, and alkylamines

[Handling Precautions]

Avoid contact with moisture.
Store sealed under inert atmosphere in a fridge.
Do not inhale vapor.
Avoid contact with skin, eyes and clothing.

References

L. Birkofer, M. Donike, *J. Chromatogr.* **1967**, *26,* 270.

M0672 *N*-Methyl-*N*-trimethylsilyltrifluoroacetamide (=MSTFA) 5 mL 25 mL

[Application Example]

MSTFA is more volatile than BSTFA and BSA.[1)] As its byproduct *N*-methyltrifluoroacetamide presents a further shorter retention time than MSTFA, overlapping of the peaks can be avoided. MSTFA works more effectively than BSTFA and BSA in the trimethylsilylation of steroids.[2)] Amine hydrochlorides can be directly trimethylsilylated.

References

1) M. Donike, *J. Chromatogr.* **1969**, *42*, 103.
2) H. Gleispach, *J. Chromatogr.* **1974**, *91*, 407.

T0492 *N*-(Trimethylsilyl)diethylamine (=TMS-DEA) 25 mL
T0591 *N*-(Trimethylsilyl)dimethylamine (=TMS-DMA) 25 mL

[Application Example]
Amino acid[1-4)]

100 mol% excess of TMS-DEA or TMS-DMA (usually 1.5-2.0 mL) is added to a sample and is heated to reflux, which subsequently resulted in a transparent solution (It is preferable to remove the resulting diethylamine or dimethylamine by distillation). After cooling, it is diluted with benzene to a proper concentration to use directly as a GC sample. If a catalytic amount of TMCS or trichloroacetic acid is added, better results are acquired. This method is also applicable to samples other than amino acids.

Fatty acids in urine

0.15 mL of either TMSDEA or TMSDMA and 0.1 mL of TMCS is added to a trace amount of a sample in dry pyridine (0.1 mL), and then the mixture is left at room temperature.

References

1) E. D. Smith, H. Sheppard, *Nature* **1965**, *208*, 878.
2) K. Rühlmann, W. Giesecke, *Angew. Chem*. **1961**, *73*, 113.
3) P. S. Mason, E. D. Smith, *J. Gas Chromatogr*. **1966**, *4*, 398.
4) E. D. Smith, K. L. Shewbart, *J. Chromatogr. Sci.* **1969**, *7*, 704.

T0585 TMS-Imidazole (=SIM, *N*-Trimethylsilylimidazole) 25 g 100 g
T0693 TMS-Imidazole Kit (=SIM Kit)

Contents of the Kit: reagent (1 mL) vial × 8, 2 mL blank vial × 8

[Application]

Reacts only with hydroxyl groups, sugars, steroids, and uric acids.

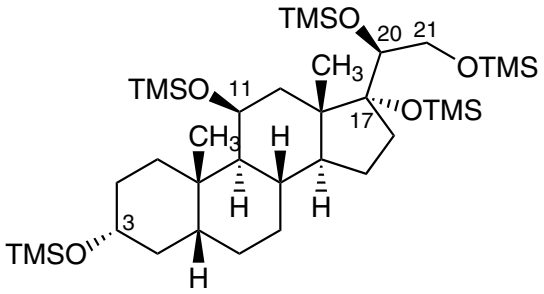
[Application Example]

Steroids

0.2 mL of SIM (*N*-TMS-imidazole) is added to 1-5 mg of substrate in 0.1 mL of pyridine. After sealing a vial, the mixture is left for 0.5-1 h at room temperature. The mixture can usually be used as a GC sample. In the case of applying to a sterically hindered hydroxyl group, it is recommended to use BSA and TMCS together with SIM. For ketosteroids, after leaving 3 h at room temperature, 0.2 mL of BSA is added and is left for further 2 h at room temperature. The resulting transparent solution can be used for GC. By this method, carbonyl groups are converted into enol TMS ethers and these derivatives are very useful for GC. Furthermore, the reaction improves by adding a trace of TMCS.

Cortol[5)]

Trimethylsilylation of cortol with 3:3:2 volume mixture of SIM, BSA and TMCS affords penta-TMS derivative cortol. If BSA is used alone, the hydroxyl groups at 3-, 20-, and 21-position are silylated. When BSA and TMCS are used together, the hydroxyl groups at 3-, 11-, 20-, and 21-position are trimethylsilylated.



Trimethylsilylation by SIM, BSA, and TMCS

[Application Example]

Avoid contact with moisture.
Store sealed under inert atmosphere in a fridge.
Do not inhale vapor.
Avoid contact with skin, eyes and clothing.

References

1) M. G. Horning, A. M. Moss, E. C. Horning, *Biochem. Biophys. Acta* **1967**, *148*, 597.
2) Y. Masada, K. Hashimoto, T. Inoue, T. Sawada, *YAKUGAKU ZASSHI* **1969**, *89*, 734.
3) E. C. Horning, M. G. Horning, N. Ikekawa, E. M. Chambaz, P. I. Jaakonmaki, C. J. W. Brooks, *J. Gas Chromatogr.* **1967**, *5*, 283.
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5) E. M. Chambaz, E. C. Horning, *Anal. Lett.* **1967**, *1 (3),* 201.
6) M. G. Horning, A. M. Moss, E. A. Boucher, E. C. Horning, *Anal. Lett.* **1968**, *1*, 311.
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T0623 TMS-PZ **12 mL**
T0692 TMS-PZ Kit **Contents of the Kit: reagent (1 mL) vial × 8, 2 mL blank vial × 8**

TMS-PZ is useful for the trimethylsilylation of aqueous sugar solutions. Although trimethylsilylating reagents normally need to be used under dried conditions, TMS-PZ can be used in aqueous sugar solutions.

[Application Example]

10% aqueous solution of sugar (5-10 µL) is poured into 1 mL of TMS-PZ. After the generation of slight heat, the mixture is shaken for 30 s, and is left for 5 min at room temperature or heated to 60-70 °C (e.g. for raffinose, it is heated to 60-70 °C (bath temperature) for 15 min). The resulting clear solution is directly injected into GC.

Note: If a liquid is poured into the sealed reaction vial in the kit, it is recommended to reduce air pressure of the vial in advance by using a syringe.

[Handling Precautions]

Store under inert atmosphere in a fridge.
Do not inhale vapors.
Avoid contact with skin, eyes and clothing.

Dimethylsilylating Reagents

C0778 Chlorodimethylsilane (=DMCS) **25 mL 250 mL**

These reagents are for the preparation of dimethylsilyl ethers, which are more volatile than TMS ethers. TMDS and DMCS (as a catalyst) are used together.

[Handling Precautions]

DMCS is decomposed by moisture to emit hydrogen chloride gas.

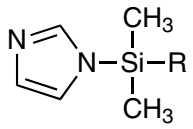
References

1) W. R. Supina, *et al*., *J. Am. Oil Chem. Soc.* **1967**, *44*, 74.
2) W. J. A. Vanden Heuvel, *J. Chromatogr.* **1967**, *27*, 85.
3) W. W. Wells, *et al*. in "*Biomedical Applications of Gas Chromatography*." *H. A. Szymanski, Ed., Plenum Press, New York.* **1964**, 199.

Dimethylalkylsilylating Reagents

D1516	**1-(Dimethylethylsilyl)imidazole**	**1 g**	**5 g**
D1596	**1-(Dimethylisopropylsilyl)imidazole**	**1 g**	**5 g**
B1043	**1-(*tert*-Butyldimethylsilyl)imidazole**	**1 g**	**5 g**

Dimethylalkylsilylating reagents are used for structural analysis of hydroxysteroids by GC-MS and for analysis of prostaglandins, bile acids, and thromboxane.



R: C_2H_5 (D1516)
$CH(CH_3)_2$ (D1596)
$C(CH_3)_3$ (B1043)

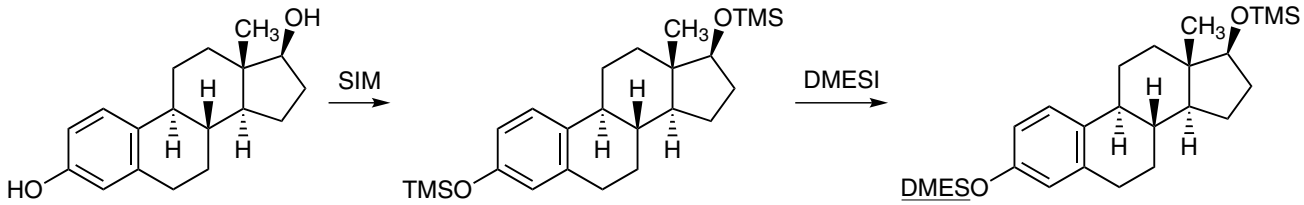
In Gas Chromatography-Mass Spectrometry (GC-MS) of hydroxysteroids, trimethylsilylating reagents such as *N*-trimethylsilylimidazole (SIM) are used in the preparation of derivatives. However, it is sometimes difficult to distinguish between alcoholic hydroxyl group and phenolic group when analyzing the structures of unknown compounds.

The dimethylalkylsilylating (DMAS) reagents have been studied and developed to improve upon the disadvantages of SIM.[1-3,8)]

The DMAS reagents are prepared by replacing one of methyl group of SIM with an alkyl group. The reaction with hydroxyl groups proceeds rapidly at room temperature similarly to TMS reagents (If the sample has a sterically hindered hydroxyl group, the reaction needs to be heated to 100 °C).

The DMAS ethers are generally more stable than the corresponding TMS ethers and also show better separation resolution in GC. The number of hydroxyl groups can be detected by comparing the methylene unit (MU) with trimethylsilylated compounds. This facilitates an accurate structural analysis of steroids by MS. It is also used for the trace analysis of biological samples such as prostaglandins[6,9,11-13)] and bile acids.[4,5,7,10)]

Trimethylsilyl ethers from the phenolic hydroxyl group have a characteristic to exchange to DMAS ethers, and vice versa in GC by "sandwich injection". This reactivity enables us to distinguish between alcoholic hydroxyl groups and phenolic groups by GC-MS.



Exchange reaction of β–estradiol from a TMS group to a DMES group

The following is an example of silylation (sandwich injection) of β–estradiol by 1-(Dimethylethylsilyl)imidazole (DMESI).

[Application] (Sample: β–estradiol)

Ⅰ. Procedure of the exchange reaction from TMS to DMES by "Sandwich injection"

1. Preparation of β–estradiol bis-TMS ether: 0.1 mg of β–estradiol is prepared in a sealable vial and is dissolved in 20 µL of SIM. The mixture is allowed to react for 30 min at room temperature.
2. Sandwich injection: 0.2 µL of DMESI, 0.1 µL of the mixture prepared in procedure 1 and 0.2 µL of DMESI are taken with a microsyringe successively, and then injected into the GC in one shot.

II . Gas Chromatogram

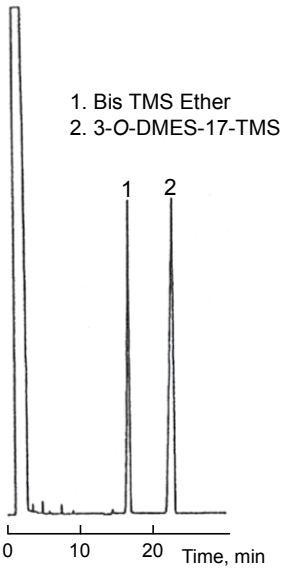


Figure 1. TMS→DMES

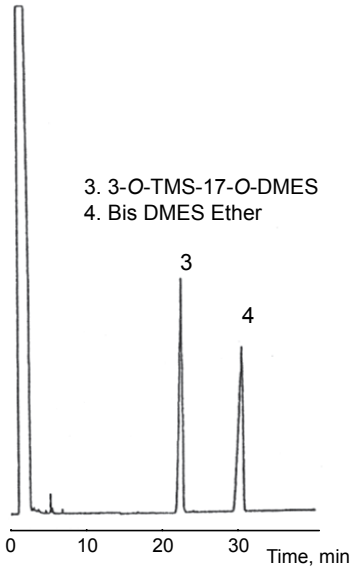


Figure 2. DMES→TMS

GC Condition of Figure 1. and Figure 2.

Column	:	007-1
		25 m × 0.53 mm I. D. × 1 μm
Temperature	:	250 °C
Detector	:	FID:23 × 27, Inj.:300 °C,
		Splitless Injection
Carrier Gas	:	He 0.3 kg/cm^2, 30 cm/s

References

1) H. Miyazaki, M. Ishibashi, M. Itoh, T. Nambara, *Chem. Pharm. Bull.* **1975**, *23*, 3033.
2) H. Miyazaki, M. Ishibashi, M. Itoh, T. Nambara, *Biomed. Mass Spectrom.* **1977**, *4*, 23.
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5) H. Miyazaki, M. Ishibashi, K. Yamashita, *Biomed. Mass Spectrom.* **1978**, *5*, 469.
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12) H. Miyazaki, M. Ishibashi, K. Yamashita, Y. Nishikawa, M. Katori, *Biomed. Mass Spectrom.* **1981**, *8*, 521.
13) H. Miyazaki, *et al*., *J. Chromatogr.* **1982**, *239*, 595.
14) Y. Harada, H. Miyazaki, *et al*., *Prostaglandins* **1982**, *23*, 881.

Related Products

D0135	Dimethylethylchlorosilane		5 g	25 g
D1590	Chlorodimethylpropylsilane		5 mL	25 mL
D1594	Dimethylisopropylchlorosilane		5 mL	25 mL
B0995	*tert*-Butyldimethylchlorosilane	5 g	25 g	100 g
T0585	*N*-Trimethylsilylimidazole		25 g	100 g
B1150	*N*-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide		1 g	10 g

tert-Butyldimethylsilylating Reagents

B1150 *N*-(*tert*-Butyldimethylsilyl)-*N*-methyltrifluoroacetamide (=MTBSTFA) 1 g 10 g

MTBSTFA is used for *tert*-butyldimethylsilylation of hydroxyl group, carboxyl group, thiol group and amino group.

tert-Butyldimethylsilylated (TBDMS or TBS) derivative is widely used for synthesis of natural products and GC-MS analysis because of its relative stability in the presence of water and highly reactive reagents (e.g. Wittig reagents, CrO_3, RMgX and RLi) and its ease of handling.

The TBDMS-Cl / Imidazole / DMF reaction conditions[1)] are generally applied when introducing *tert*-butyldimethylsilyl group. However, it is chellenging to *tert*-butyldimethylsilylate thiol groups, amino groups, and sterically hindered hydroxyl groups. Fortunately, MTBSTFA is an effective silylating agent for these functional groups. The reaction can be completed in 5-20 min at room temperature in most cases, and the reaction mixture can directly be injected into GC.

MTBSTFA is used for GC or GC-MS analysis of thiols,[2,15)] amines,[2)] polyamines,[5)] amino acids,[2,6,8,9)] dipeptides,[11)] ketone bodies,[6,7)] fatty acids,[6,10,13,16)] hydroxyeicosatetraene acids,[12,14)] leucotrienes[12)] and alkylphosphonic acids,[17)] and also is used for GC-MS analysis of prostaglandins[3)] and oxygen-containing anions.[4)]

References

1) E. J. Corey, *et al*., *J. Am. Chem. Soc*. **1972**, *94*, 6190.
2) T. P. Mawhinney, *et al*., *J. Org. Chem*. **1982**, *47*, 3336.
3) A. C. Bazan, *et al*., *J. Chromatogr.* **1982**, *236*, 201.
4) T. P. Mawhinney, *J. Chromatogr.* **1983**, *257*, 37.
5) N. G Lay-Keow, *J. Chromatogr.* **1984**, *314*, 455.
6) W. F. Schwenk, *et al*., *Anal. Biochem*. **1984**, *141*, 101.
7) J. M. Miles, *et al*., *Anal. Biochem.* **1984**, *141*, 110.
8) C. J. Biermann, *et al*., *J. Chromatogr.* **1986**, *357*, 330.
9) T. P. Mawhinney, *et al*., *J. Chromatogr.* **1986**, *358*, 231.
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11) M. E. Corbett, *et al*., *J. Chromatogr.* **1987**, *419*, 263.
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15) D. C. Landrum, T. P. Mawhinney, *J. Chromatogr.* **1989**, *483*, 21.
16) K. R. Kim, *et al*., *J. Chromatogr.* **1989**, *468*, 289.
17) J. G. Purdon, *et al*., *J. Chromatogr.* **1989**, *475*, 261.

Related Products

B1043	1-(*tert*-Butyldimethylsilyl)imidazole	1 g	5 g
A1275	Allyldimethylsilyl Chloride	10 mL	25 mL

Halomethyldimethylsilylating Reagents [for GC-ECD]

B0990 1,3-Bis(chloromethyl)tetramethyldisilazane 5 g
[Application] Acids,[2] phenols,[2] steroids[1,3] and sugars. Used together with CMDMCS.

C0605 (Chloromethyl)dimethylchlorosilane (=CMDMCS) 25 g 250 g
B0847 (Bromomethyl)dimethylchlorosilane (=BMDMCS) 25 g
[Application] Acids,[2] phenols[2] and steroids.[1]

Halomethylsilylating reagents are highly effective when detecting trace amounts of components by an Electron Capture Detector (ECD).

[Application]
How to use halomethyldimethylsilyldiethylamine solution[1,2]

1 mL of hexane, 0.075 mL of diethylamine and 0.09 mL of (halomethyl)dimethylchlorosilane are mixed in a sealable vessel and centrifuged. 0.4 mL of the resulting supernatant is added to 100 µg of sample in 0.1 mL of ethyl acetate and refluxed for 30 min at 65 °C. The mixture is then promptly cooled to room temperature followed by adding hexane to adjust to the appropriate concentration. This solution is injected into GC.

References

1) C. Eaborn, D. R. M. Walton, *Chem.& Ind.* **1967**, 827.
2) C. A. Bache, L. E. St. John, D. J. Lisk, *Anal. Chem.* **1968**, *40*, 1241.
3) B. S. Thomas, D. R. M. Walton, *"The Gas Liquid Chromatography of Steroids"* ed. by J. K. Grant p199.

Pentafluorophenyldimethylsilylating Reagents [for GC-ECD]

P0908 Pentafluorophenyldimethylsilyldiethylamine (=Flophemesyldiethylamine) 100 mg
P0854 Pentafluorophenyldimethylchlorosilane (=Flophemesyl Chloroide) 1 mL 5 mL

[Application]
Alcohols

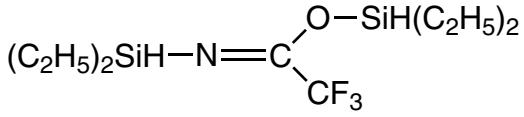
The substrate (primary alcohol) is dissolved in pyridine and 1:1 mixture of pentafluorophenyldimethylsilyldiethylamine and pentafluorophenyldimethylchlorosilane is subsequently added. This mixture can be directly used for GC-ECD analysis. This can also be used for GC-MS analysis both in high selectivity and in high sensitivity. In the case of tertiary alcohols, the derivatization is completed by reacting for 10 min at 25 °C.

References

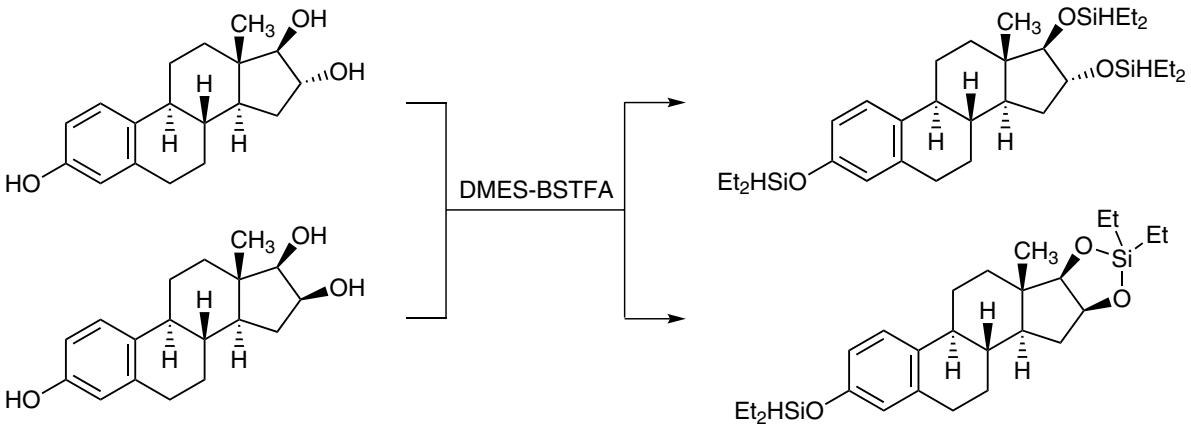
P. W. Burkinshaw, E. D. Morgan, *J. Chromatogr.* **1977**, *132*, 548.

Simultaneous cyclic silylene and silyl derivatizing reagent

B1435 *N,O*-Bis(diethylhydrogensilyl)trifluoroacetamide (=DEHS-BSTFA) 1 g

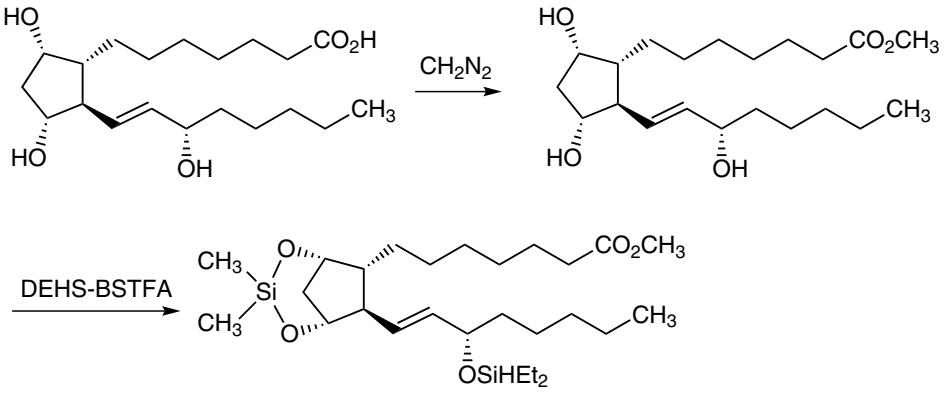


The analysis of 1,2- and 1,3- diols in GC's frequently involves their conversion to cyclic boronate or di-*tert*-butylsilylene derivatives. Nevertheless, for compounds with an isolated hydroxyl group, the hydroxyl group remains unreacted, and necessitates a secondary treatment such as an additional trimethylsilylation to achieve protection.



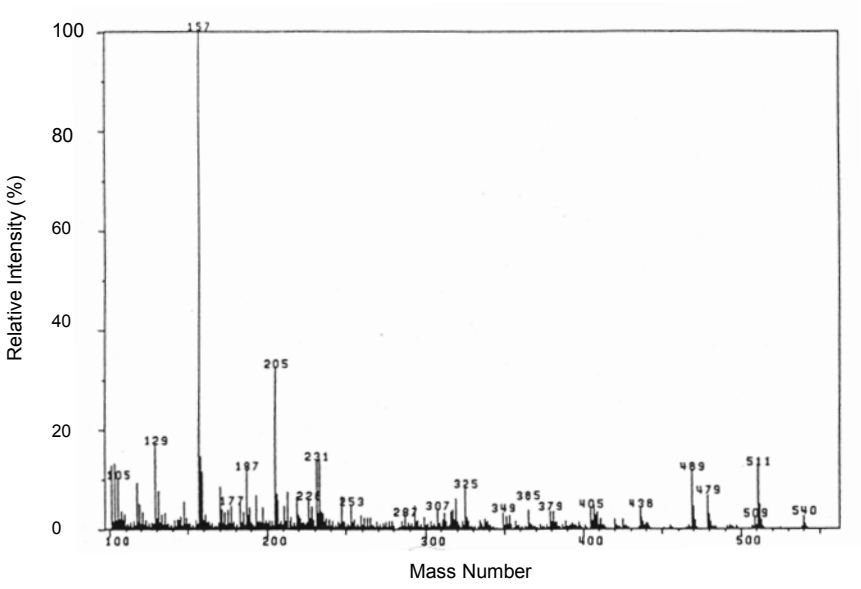
Miyazaki *et al*. have developed a single step derivatization reaction that produces both cyclic diethylsilylene (DES) from 1,2- and 1,3-diols and a diethylhydrogensilyl ether (DEHS) from a hydroxyl group by applying DEHS-BSTFA to hydroxysteroids.[1)] According to their results, as for hydroxyl groups on D rings, 1,2-*cis* diol produces cyclic DES selectively. (See above equation)

The ratio of cortisol and its metabolite 6β-hydroxycortisol in urine has been received attention as potential indicators for the function of hepatic drug-metabolizing enzymes. The MO-TMS method is generally used for ketosteroids analysis but it is not suitable for cortisol and 6β-hydroxycortisol due to difficulties encountered during separation. Ishibashi *et al*. have developed a method making it possible to simultaneously quantify the constituents in urine by converting them to MO-DEHS-DES derivatives using DEHS-BSTFA. Furthermore, Goto *et al*. have reported the use of DEHS-BSTFA as a derivatizing reagent for GC-MS analysis of abnormal bile acids containing a hydroxyl group at 4th and 6th position in fetuses and neonates.



Ishibashi *et al*. have used DEHS-BSTFA to induce F_{α}PG (e.g. prostaglandin (PG) $F_{1\alpha}$, $F_{2\alpha}$, and 6-keto $PGF_{1\alpha}$, and 13,14-dihydro-15-keto $PGF_{2\alpha}$), thromboxane (TX) B_2 and 11-dehydro TXB_2 to cyclic DES derivatives. Detailed analysis by GC/MS have indicated that the resulting cyclic DES derivatives show a characteristic mass spectrum. [3,4)]

In this way, DEHS-BSTFA is used as an effective derivatization reagent for GC-MS analysis of hydroxysteroids, bile acids, and prostaglandins.



Mass spectrum of DEHS-DES derivatives of $PGF_{1\alpha}$ Methyl Ester

References

1) H. Miyazaki, M. Ishibashi, M. Itoh, K. Yamashita, *Biomed. Mass Spectrom.* **1984**, *11*, 377.
2) M. Ishibashi, H. Takayama, Y. Nakagawa, N. Harima, *Chem. Pharm. Bull.* **1988**, *36*, 845.
4) M. Ishibashi, K. Watanabe, K. Yamashita, *J. Chromatogr.* **1987**, *391*, 183.
5) K. Watanabe, M. Ishibashi, N. Harima, S Krolik, *Chem. Pharm. Bull.* **1989**, *37*, 140.

Esterification

Acid Catalyst Anhydrous Alcohols

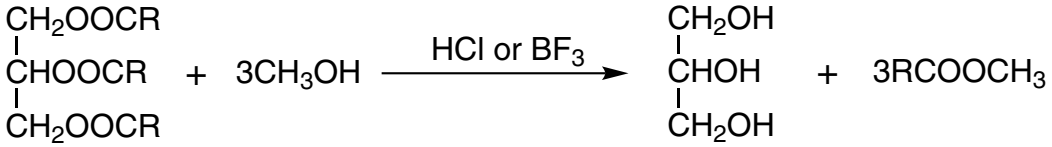
X0034	**BF_3 - Butanol Reagent** (10-20%)	**1 mL×10**
X0035	**BF_3 - Isopropanol Reagent** (10-20%)	**1 mL×10**
X0037	**BF_3 - Propanol Reagent** (10-20%)	**1 mL×10**
X0036	**BF_3 - Methanol Reagent** (10-20%)	**1 mL×10**
H0959	**HBr - Ethanol Reagent** (10-20%)	**25 mL 500 mL**
X0043	**HBr - Methanol Reagent** (5-10%)	**25 mL 500 mL**
X0039	**HCl - Butanol Reagent** (5-10%)	**1 mL×10**
X0038	**HCl - Methanol Reagent** (5-10%)	**1 mL×10**
X0041	**HCl - Methanol Reagent** (5-10%)	**25 mL 500 mL**

[**Application**]

Experimental procedures differ from types of esterification reagents or purposes. Typical applications are shown below. Please refer to the references for details.

[General Procedures]

- 500 mg of substrate (e.g. stearic acid or linolenic acid) is placed into a test tube, and 1 mL of HCl-MeOH or BF_3-MeOH is added. After attaching a reflux condenser, the mixture is heated to reflux for about 0.5 - 1 h. Then cooled to room temperature, 1 mL of distilled water is added and followed by extraction with 1 mL of hexane. The hexane solution is directly injected into GC as a sample.
- After the esterification of trace fatty acids extracted from a biological sample, only esters will be obtained from the sample containing unsaponificated components by microsublimation.[1)]
- Free fatty acids from oil can be adsorbed onto a resin (Amberlite IRA-400) and can be directly esterified on the resin and subsequently extracted.[5)]
- When analyzing the composition of fatty acids in glycerides, esterification of free fatty acids (obtained by saponification) can be applicable. However, it is more convenient to obtain esters directly by transesterification since the reaction occurs in one step.



CAUTION: Wear appropriate PPE and open reaction vessles with extreme care after cooling, as it irritates the eyes, skin and bronchitis, and is also corrosive and may still be under pressure. Store in a cool place to avoid an increase in internal pressure of the container.

References

1) Esterification with HCl-alkanol W. Stoffel, *Anal. Chem*. **1959**, *31*, 307.
2) Esterification with BF_3-alkanol L. D. Metcalfe, *Anal. Chem*. **1961**, *33*, 363.
3) Ester interchange with HCl-alkanol M. E. Mason, *Anal. Chem*. **1964**, *36*, 583.
4) Ester interchange with BF_3-alkanol F. E. Luddy, *J. Am. Oil Chem. Soc*. **1968**, *45*, 549.
5) Esterification of absorbed fatty acid on resin Hornstein, *Anal. Chem*. **1960**, *32*, 540.
6) Esterification with BCl_3-2-Chloroethanol D. D. Woodhem, *J. Agr. Food Chem*. **1971**, *19*, 186.

N,*N*-Dimethylformamide Dialkylacetals

D2071	***N*,*N*-Dimethylformamide Dimethyl Acetal**	**25 mL**
D1332	***N*,*N*-Dimethylformamide Dimethyl Acetal**	**0.5 mL×10**
D1294	***N*,*N*-Dimethylformamide Diethyl Acetal**	**5 mL 25 mL**
D1301	***N*,*N*-Dimethylformamide Dipropyl Acetal**	**5 mL 25 mL**
D1302	***N*,*N*-Dimethylformamide Dibutyl Acetal**	**5 mL 25 mL**
D1303	***N*,*N*-Dimethylformamide Di-*tert*-butyl Acetal**	**5 mL 25 mL**
D1595	***N*,*N*-Dimethylformamide Dineopentyl Acetal**	**5 mL 25 mL**

The listed compounds (except *N*,*N*-Dimethylformamide Dineopentyl Acetal) act as esterification reagents for fatty acids and can readily provide the corresponding alkyl esters. In addition, these reagents can be used to convert amino acids into the corresponding *N*-dimethylaminomethylene-*O*-alkyl esters in one step. These dimethylformamide acetals are liquid at room temperature, are easy to handle, and are stable at room temperature as long as stored away from moisture.

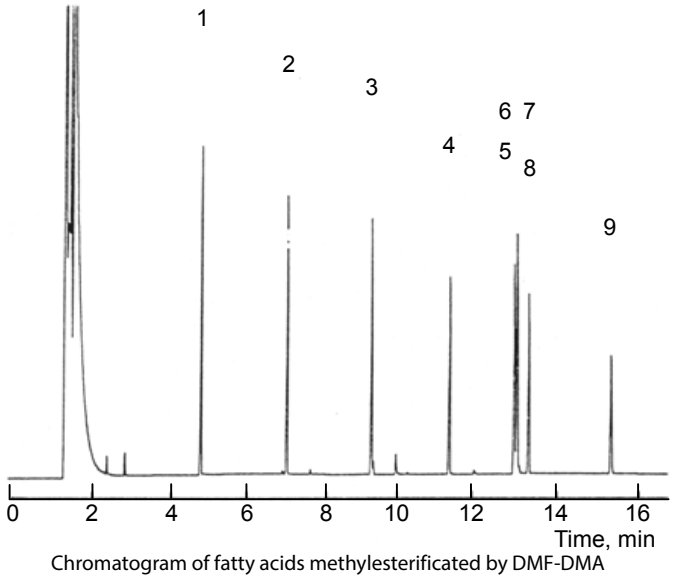
1. Esterification of fatty acids[1)]

$$R'COOH + (CH_3)_2NCH(OR)_2 \longrightarrow R'COOR + ROH + HCON(CH_3)_2$$

[Application Example]

5 mg of fatty acid is placed into a vial and then 100 µL of an esterification reagent is added. The reaction is completed upon dissolution. The reaction mixture can be injected directly into GC.

Using this method, after washing with water, extraction and condensation procedures is generally not required. In addition, water is not produced as a byproduct during the reaction. If the sample is a solid with long carbon chains, a solvent can be added and heated slightly. The reaction time can be shortened for the completion if some samples are dissolved in a variety of solvents (e.g. pyridine, benzene, methanol, chloroform, dichloromethane, THF, DMF, etc.) because these reagents cannot be used as proper solvents.



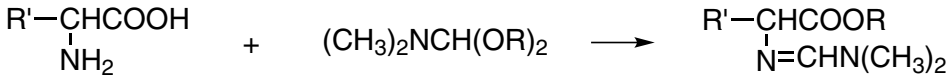
Chromatogram of fatty acids methylesterificated by DMF-DMA

1. C_{10}
2. C_{12}
3. C_{14}
4. C_{16}
5. $C_{18:2}$
6. $C_{18:1}$
7. $C_{18:3}$
8. C_{18}
9. C_{20}

GC Condition

Column : 007-1,
25 m × 0.25 mm I. D. × 0.25 µm
Temperature : 100 °C~(10 °C/min)~240 °C
Detector : FID: $2^3 \times 2^5$
Injection : 300 °C
Carrier Gas : He: 0.9 kg/cm^2, 30 cm/s

2. Reaction with amino acids[2)]



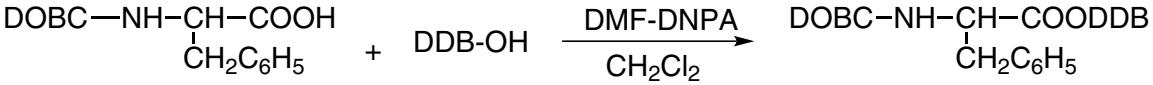
[Application Example]

The reaction is completed when the reaction mixture becomes a solution. Although various reaction solvents can be used, acetonitrile is the most recommended for this reaction. Most amino acids react in acetonitrile solution (1:1) and the reaction is completed at 100 °C for 20 min, while aspartic acid requires longer reaction time.

An *N*-dimethylaminomethylene alkyl ester can be obtained from an amino acid by this reaction.

3. *N,N*-Dimethylformamide Dineopentyl Acetal (=DMF-DNPA)[3, 4)]

DMF-DNPA itself does not act as an esterification reagent but mediates esterification.



DOBC=n-$C_{10}H_{21}O$-$C_6H_4CH_2O$-CO-

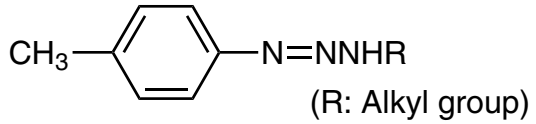
DDB=n-$C_{12}H_{25}$-C_6H_4-CH_2-

References

1) J. P. Thenot, E. C. Horning, M. Stafford, M. G. Horning, *Anal. Lett.* **1972**, *5*, 217.
2) J. P. Thenot, E. C. Horning, *Anal. Lett.* **1972**, *5*, 519.
3) A. Kirrmann, J. J. Delpuech, *Compt. Rend.* **1965**, *260*, 6600.
4) J. J. Delpuech, *Bull. Soc. Chim. France* **1966**, 1624.

1-Alkyl-3-*p*-triazenes

M0641 1-Methyl-3-*p*-tolyltriazene **1 g 25 g**
B0949 1-Benzyl-3-*p*-tolyltriazene **1 g 25 g**

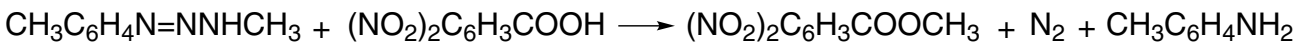


1-Alkyl-3-*p*-tolyltriazenes react with carboxylic acids rapidly under mild conditions to give the corresponding esters in high yields.[1)]

These reagents can also be used for alkylation of phenols,[2)] imides and enolized ketones.[3)] Furthermore, it has been reported that these reagents can be used for the alkylation of alcohols[3)] and thiols[4)] in the presence of a catalyst such as trimethoxyaluminium.

[Applications]

1. Methylesterification of 3,5-dinitrobenzoic acid[1b)]



25 mL of ether solution of a sample (1.50 g, 7.1 mmol) is slowly added to 10 mL of ether solution of 1-Methyl-3-*p*-tolyltriazene (1.05 g, 7.0 mmol) with occasional stirring. During solution addition, the reaction mixture turns red with the evolution of N_2. After the evolution of N_2 is completed (about 1 h), the ether solution is washed with 5M-HCl to remove the by-product toluidine. The mixture is washed with 5% sodium carbonate solution and dried over anhydrous Na_2SO_4. The ether is removed by concentration to obtain a methyl ester (1.11-1.42 g, 70-90%, light yellow-brown crystal, mp 106-107.5 °C). The residue is recrystallized from ether to give small plate crystal. (mp 107-107.5 °C). A variety of esters can be prepared from the other corresponding triazenes using this procedure.

2. Methylesterification of fatty acids and its application for GC

1 mL of 10% ether solution of 1-methyl-3-*p*-tolyltriazene is added to *ca*. 50 mg of mixture of fatty acid in a flask. The mixture is refluxed in a water bath for 30 min. After cooling, 1 mL each of hexane and HCl (1:10) are added and the mixture is shaken with periodic venting. After being left for some minutes , 1 µL of the hexane layer is injected into GC.

References

1) a) E. H. White, H. Scherrer, *Tetrahedron Lett*. **1961**, *21*, 758.
b) E. H. White, A. A. Baum, D. E. Eitel, *Org. Synth*. **1968**, *48*, 102.
c) *Ukrain. Khim. Zhur.* **1952**, *18*, 631.
2) *Ukrain. Khim. Zhur.* **1954**, *20*, 284.
3) *Ukrain. Khim. Zhur.* **1955**, *21*, 496.
4) *Ukrain. Khim. Zhur.* **1955**, *21*, 628.

On-Column Methyl Esterification

T3610	**Phenyltrimethylammonium Hydroxide (=PTAH)** (8.5% in Methanol)	**25 mL**	**100 mL**
T0676	**Tetramethylammonium Hydroxide (=TMAH)** (10% in Methanol)	**25 mL**	**500 mL**
T1576	**Trimethylsulfonium Hydroxide** (0.2mol/L in Methanol)	**5 mL**	**25 mL**

The following applications are for PTAH (8.5% in Methanol) and TMAH (10% in Methanol) as on-column methylation reagents in the sample vaporization chamber of GC's.

Methylesterification of fatty acids

Esterification by diazomethane is often carried out for GC analysis of heat-labile and relatively highly polar fatty acids. However, the reagent is difficult to handle due to its extreme toxicity and explosiveness, and the reaction often does not proceed quantitatively.

PTAH is very effective for "on-column methylation" and the reaction readily and rapidly proceeds quantitatively. Furthermore, it is safe and easy to handle. For example, Middleditch *et al*. showed efficient esterification and analysis in the separation of esterificated fatty acids.[9] Namely, 1 mg of fatty acid mixture is dissolved in 0.5 mL of 0.2 M methanol solution of PTAH at room temperature and 1 mL of the above mixture is directly injected into the sample vaporization chamber. In this reaction, it is hypothesized that phenyltrimethylammonium salts generated from the acids at room temperature, produce esters and dimethylaniline as a byproduct by thermolysis in vaporization chamber.

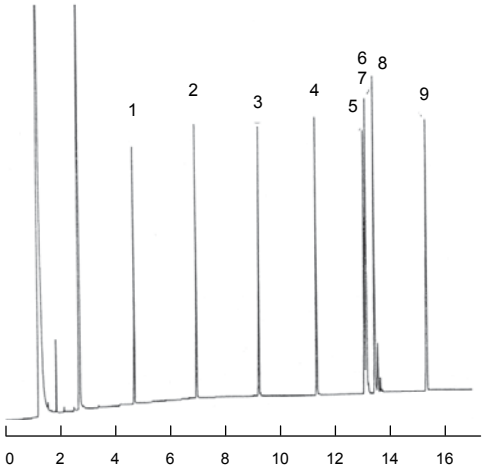
The use of TMAH includes the esterification of the carboxylic acids by Robb *et al*. [4] and the methylation of purine and pyrimidine bases.[5]

Methylation of barbituric acids

Martin *et al*.[3] have found they obtained better separation ability with sharp spectra peaks by injecting methylated barbituric acids into GC compared to injecting free acids directly.[2] However, it takes time and labor for methylation. Stevenson[1] has applied "on-column methylation" by TMAH to the analysis of barbituric acids. Namely, 1 mL of 0.1 M methanol solution of TMAH was added to each 1 mg of the acids and then the resulting mixture was partially injected into GC. They have found that the reaction proceeded quantitatively in the molar ratio 1:4, sample-reagent.

PTAH is also used as an "on-column methylation" reagent for barbituric acids,[6-8] sedatives,[6,8] xanthines,[5] phenolalkaloids,[7] diphenylhydantoin sodium salt,[8] etc. and gives good results for GC analysis.

500 µL of 0.2 M methanol solution of PTAH is added to 1 mg of fatty acid mixture and the resulting mixture is injected into the GC column.



1. C_{10}
2. C_{12}
3. C_{14}
4. C_{16}
5. $C_{18:2}$
6. $C_{18:1}$
7. $C_{18:3}$
8. C_{18}
9. C_{20}

Chromatogram of fatty acid mixture methylated by PTAH

GC Condition

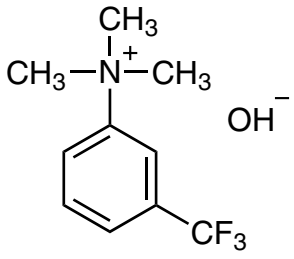
Column	:	007-1,
		25 m × 0.25 mm I. D. × 0.25 µm
Temperature	:	100 °C~(10 °C/min)~240 °C
Detector	:	FID: $2^3 \times 2^5$
Injection	:	300 °C

References

1) G. W. Stevenson, *Anal. Chem.* **1966**, *38*, 1948.
2) A. B. Svendsen, *J. Pharm. Sci.* **1962**, *51*, 318.
3) H. F. Martin, J. L. Driscoll, *Anal. Chem.* **1966**, *38*, 345.
4) E. W. Robb, J. J. Westbrook, *Anal. Chem.* **1963**, *35*, 1644.
5) J. MacGee, *Anal. Biochem.* **1966**, *14*, 305.
6) *Chemical&Engineering News* **1971**, April 12, p.13.
7) E. Brochmann-Hanssen, T. O. Oke, *J. Pharm. Sci.* **1969**, *58*, 370.
8) M. J. Barrett, The Clinical Chemistry Newsletter p.3, No.1, Spring (1971). (published by the Perkin-Elmer Corp.)
9) B. S. Middleditch, D. M. Desiderio, *Anal. Letters* **1972**, *5*, 605.

Methyl Esterification for GC

T0961 3-(Trifluoromethyl)phenyltrimethylammonium Hydroxide (=*m*-TFPTAH) (5% in Methanol) 25 mL



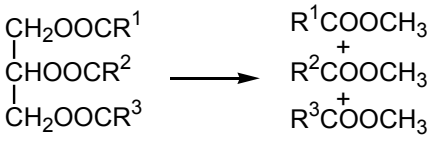
3-(Trifluoromethyl)phenyltrimethylammonium Hydroxide is used as an ester exchange reagent for triglycerides and others. It can used for the detection of triglyceride-constituent fatty acids by GC.

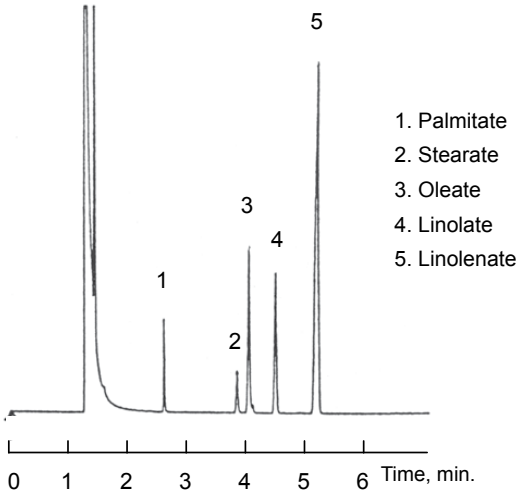
By injecting the mixture of *m*-TFPTAH and triglyceride into GC, chromatogram of methyl esters of triglyceride-constituent fatty acids can be obtained quantitatively. GC analysis of triglyceride-constituent fatty acids becomes substantially easier compared with a conventional methyl esterification method such as using sodium methoxide. *m*-TFPTAH reagent is easy to use and reacts with fatty acids without affecting double bonds in them. It also can be used as an on-column methylation reagent for fatty acids.[1,2)]

[Application]

Transesterification of linseed oil

10 mg of linseed oil in a vial is dissolved with 0.5 mL of toluene. Next, a 200 µL of 5% methanol solution of *m*-TFPTAH is added. The vial is tightly closed and left for 15 min at room temperature. 1 µL of the reaction mixture is directly injected into GC.





1. Palmitate
2. Stearate
3. Oleate
4. Linolate
5. Linolenate

Column : 007-23
0.25 mmI.D. × 25 m × 0.25 µm
Temperature : 180 °C

Capillary gas chromatogram of methyl esters of fatty acids from linseed oil

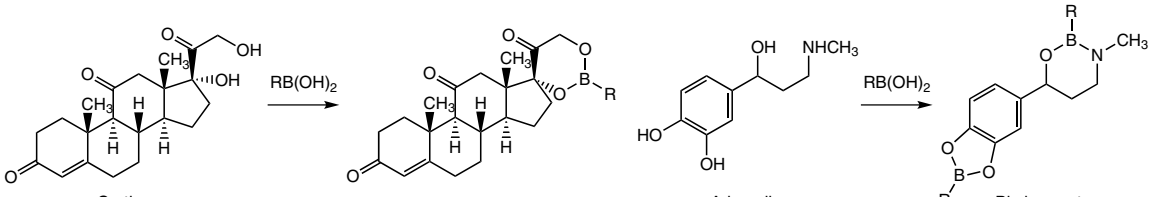
References

1) W. C. Kossa *et al.*, *J. Chromatogr. Sci.* **1979**, *17*, 177.
2) J. MacGee, K. G. Allen, *J. Chromatogr.* **1974**, *100*, 35.

Reagents for Cyclic Boronate Ester

B0529 Butylboronic Acid (contains varying amounts of Anhydride) **1 g 5 g 25 g**
B0857 Phenylboronic Acid (contains varying amounts of Anhydride) **5 g 25 g 250 g**

These reagents readily react with diols, hydroxy acids and hydroxy amines at room temperature or by slight warming to generate cyclic boronates. They can be used for GC or GC-MS analysis of hydroxy acids (e.g. tartaric acid, lactic acid, salicylic acid), catecholamines, corticosteroids, and brassinolide.



Cortisone — Adrenaline (Epinephrine) — Bis-boronate

[Application]
Corticosteroid[1,2)]

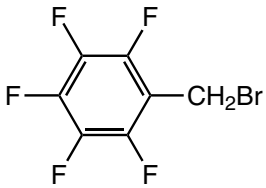
10 µmol each of steroids and butyl boronic acid are dissolved in 1 mL of ethyl acetate and the mixture is allowed to react for 5 min at room temperature.

References

1) C. J. W. Brooks, *et al*., *J. Chromatogr.* **1971**, *54*, 193.
2) C. J. W. Brooks, *et al*., *J. Chromatogr. Sci.* **1971**, *9*, 18.

Pentafluorobenzyl Bromide [for GC-ECD]

P0809 Pentafluorobenzyl Bromide **1 g 5 g 25 g**



[Application]

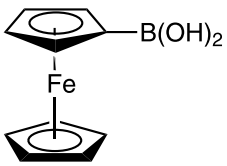
For carboxylic acids, phenols,[1)] sulfonamides,[2)] thiols and organic acids.[3-6)]

References

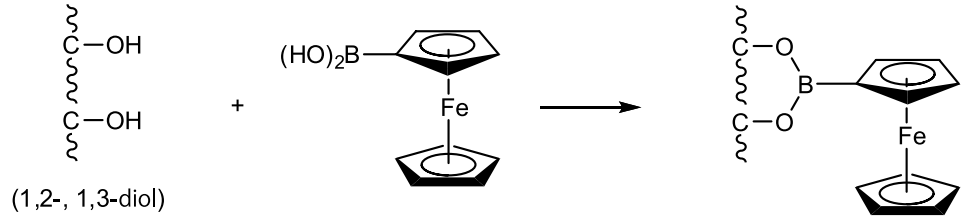
1) H. Ehrsson, *Acta Pharmaceutica Suecica* **1971**, *8*, 113.
2) O. Gylledhaal, H. Ehrsson, *J. Chromatog*. **1975**, *107*, 327.
3) F. K. Kawahara, *Anal. Chem*. **1968**, *40 (6)*, 1009.
4) F. K. Kawahara, *Anal. Chem*. **1968**, *40 (13)*, 2073.
5) F. K. Kawahara, *Environ Sci. & Tech*. **1971**, *5 (3)*, 235.
6) F. K. Kawahara, *Environ Sci. & Tech*. **1976**, *10 (8)*, 761.

Ferroceneboronic Acid

F0280 Ferroceneboronic Acid (contains varying amounts of Anhydride) **100 mg** **1 g**



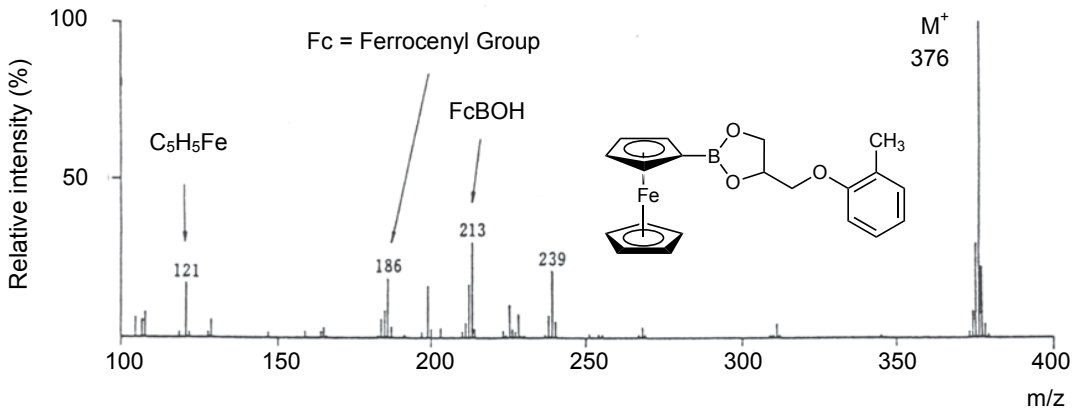
Alkyl boronic acids react with bifunctional substrates to form cyclic boronates, and are used for both GC and GC-MS analysis. The ferroceneboronic acid is a useful derivatizing reagent especially for GC-MS analysis.



Brooks *et al.* have reported that cyclic boronate derivatives give a characteristic spectrum in the Electron Impact (EI) MS (an example is shown in the figure below). The derivatives show strong molecular ion peaks and isotope peaks derived from the isotope atoms such as ^{10}B, ^{54}Fe and ^{57}Fe, which consequently facilitate the identification of bifunctional substrates. Moreover, major fragment ions are derived from reagent molecules, not from sample molecules (in the figure, m/z 239, 213, 186 and 121). Therefore, it is suitable for mass chromatography by SIM.

[General method for cyclic boronate derivatives]

100 µL of substrate is dissolved in dry pyridine. 1.1 equiv. of ferroceneboronic acid is dissolved in dry pyridine and added to the sample solution. The reaction mixture is allowed to react at 70 °C for 30 min and then pyridine is removed by nitrogen gas flow. The resulted mixture is dissolved in 100 µL of ethyl acetate and used as a sample for GC or GC-MS.



Mass spectrum of mephenesin cyclic boronate derivative

References

C. J. W. Brooks, W. J. Cole, *J. Chromatogr.* **1986**, *362*, 113.

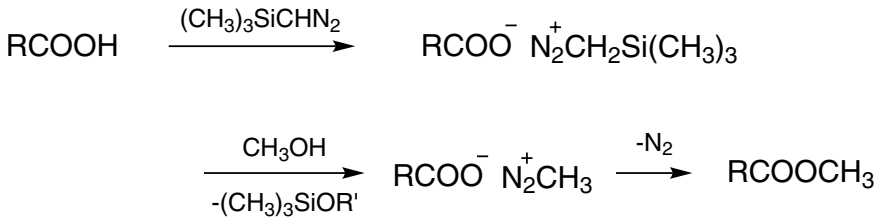
Safe Methyl Esterification Reagent

T1146 Trimethylsilyldiazomethane (=TMS-Diazomethane) (*ca*. 10% in Hexane, *ca*. 0.6 mol/L)

10 mL 25 mL 100 mL

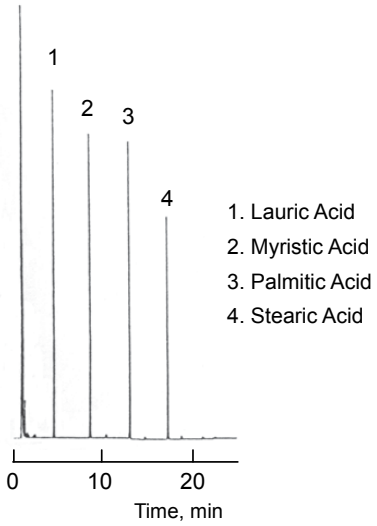
Diazomethane has long been used as a standard reagent for methyl esterification. However, it has many disadvantages including its high toxicity (e.g. acute and carcinogenic) and explosion hazard, and it also requires a detailed preparation before use.

On the contrary, TMS–diazomethane has low toxicity. Moreover, it can form methyl esters from various kinds of carboxylic acids quickly and quantitatively in the presence of methanol.



[Application]

0.1 mmol of fatty acids is dissolved in 1 mL of benzene containing 20% methanol and then 0.5 mL of this reagent is added. The mixture is stirred vigorously and left at room temperature for 30 min and used as a GC sample.



GC Condition

Column : 007-5
25 m × 0.25 mm I. D. × 0.25 µm
Temperature : 150 °C → 250 °C (5 °C/min)
Carrier Gas : He 30 cm/s

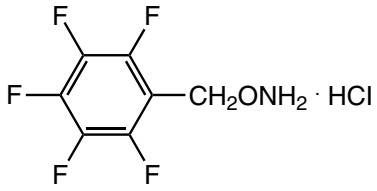
References

N. Hashimoto, T. Aoyama, T. Shioiri, *Chem. Pharm. Bull.* **1981**, *29*, 1475.

Other Pretreatment

Reagent for Preparation of Ketosteroid Oxime
For Electron Capture Detector (ECD)

P0822 *O*-(2,3,4,5,6-Pentafluorobenzyl)hydroxylamine Hydrochloride 1 g 5 g



O-(2,3,4,5,6-Pentafluorobenzyl)hydroxyamine hydrochloride (*O*-PFBHA·HCl) is an oxime derivatizing reagent used to detect trace amount of ketosteroids such as testosterone and progesterone by GC analysis with an electron capture detector (ECD). [1,2]

GC analysis with ECD has been extensively carried out for the analysis of steroids in biological tissue. However, only a few steroids have sufficient electron captivity and thus a variety of derivatizing reagents with electron capture groups have been studied and developed in order to increase the detection sensitivity. Although perfluorocarboxylic chlorides or anhydrides[3] are commonly used as esterification reagents for this purpose, they produce strong acids as a byproduct, which also reacts with steroids. In addition, it is known that incorrect recognition in analysis can occur since one steroid can often form a number of isomeric derivatives, resulting in multiple peaks. Pentafluorophenylhydradine[4,5] has a disadvantage with the thermostability of its derivatives formed on steroids are not sufficient.

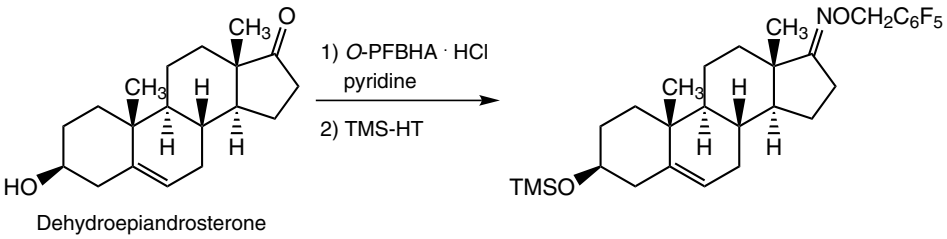
O-PFBHA·HCl is a novel derivatizing reagent for ketosteroids that solves the above-mentioned disadvantages. It reacts with traceketosteroids (1-5 ng) under a mild conditions and affords pentafluorobenzyloxime (*O*-PFBO) derivatives with few by-products. The resulting oximes have high heat stability and also excellent sensitivity to the ECD. For example, the sensitivity is 5 pg (5×10^{-12} g) for testosterone and 1 - 0.1 ng for other steroids.

The excess reagent can be easily removed by washing with acid and the unreacted hydroxyl groups in steroids become ready for GC analysis by trimethylsilylation.

Below application is the analysis of dehydroepiandrosterone extracted from human serum.

[Application][1]

An extract from serum containing epiandrosterone acetate (approx. 1 µg, as an internal standard) is dissolved in 2 drops of pyridine. *O*-PFBHA·HCl (0.2 mg) is added to the mixture and is allowed to react for 1h at 60 °C. After diluting with 3 mL of hexane, the mixture is washed with water (1 mL), 0.1 mol/L HCl (1 mL), 0.1 mol/L aqueous solution of sodium hydroxide (1 mL), and water (1 mL), followed by centrifugation. And then hexane is evaporated to obtain the residue (*O*-PFBOs). After that, the hydroxyl group is trimethylsilylated with TMS-HT. It is evaporated and dried, and then the residue is dissolved in 1 mL of hexane and 2 µL of the mixture is injected into GC.



Dehydroepiandrosterone

References

1) T. Nambara, K. Kigasawa, T .Iwata, M. Ibuki, *J. Chromatogr.* **1975**, *114*, 81.
2) K. T. Koshy *et al*., *J. Chromatogr. Sci.* **1975**, *13*, 97.
3) P. G. Devaux, E.C. Horning, *Anal. Lett*. **1969**, *2*, 637.
4) J. Attal *et al*., *Anal. Biochem*. **1967**, *20*, 394.
5) R. A. Mead *et al*., *J. Chromatogr. Sci.* **1969**, *7*, 554.

Derivatizing Reagent for GC of Inorganic Anions

T1204 Pentafluorobenzyl *p*-Toluenesulfonate (=PFB-Tosylate) 5 g

Pentafluorobenzylation using T1204 allows for the analysis of inorganic anions (Br^-, I^-, CN^-, S_2^-, NO_2^-, NO_3^-, SCN^-) by GC. Moreover, using ECD as a detector allows for highly sensitive analyses of trace amount of inorganic anions. This reagent can be used for GC analysis of pentafluorobenzylation of carboxylic acids, phenols and others.

[Application Example]

1 mL of a sample, 0.2 mL of 0.1 mol/L aqueous solution of tetra-*n*-amylammonium chloride (TAAC), and 1 mL of 0.1 mol/L dichloromethane solution of this reagent are placed in a screw capped 10 mL brown bottle, and it is tightly sealed. After stirring for 30 min, the lower layer of the mixture is injected into GC.

Measuring Range

Anions	Derivatives	Measuring Range (FID)
Bromide	PFB-Bromide	30～300 ppm
Cyanide	PFB-Cyanide	10～100 ppm
Iodide	PFB-Iodide	50～500 ppm
Nitrite	PFB-Nitrite	45～450 ppm
Nitrate	PFB-Nitrate	25～250 ppm
Sulfide	PFB-Sulfide	6.5～65 ppm
Thiocyanate	PFB-Thiocyanate	20～200 ppm

References

K. Funazo, *et al*., *J. Chromatogr.* **1985**, *346*, 215.

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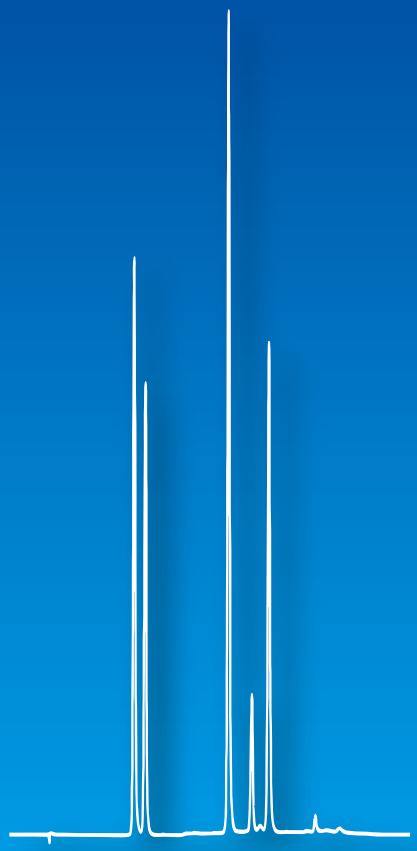
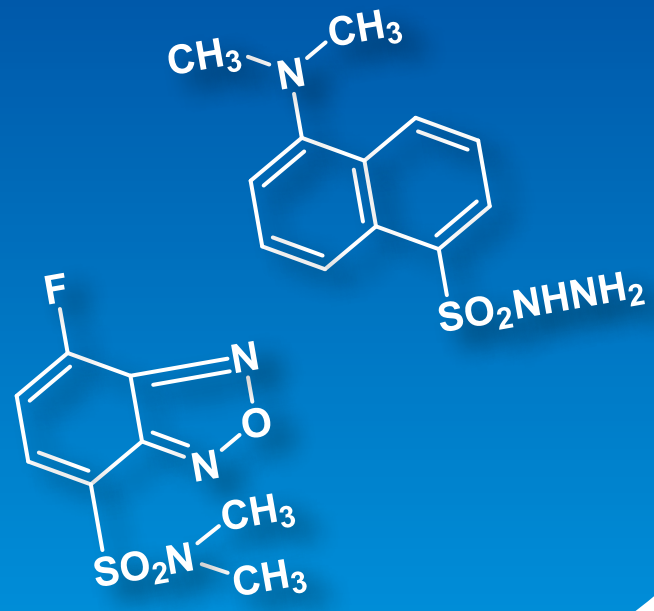
HPLC Labeling Reagents





HPLC Labeling Reagents

HPLC is utilized extensively as a means of detecting and determining trace components. Labeling objective substances for analysis with labeling reagents appropriate for detection methods has been performed in order to obtain higher sensitivity and selectivity. Many labeling reagents have been reported for this purpose. We picked up a part of them and sell them as our TCI-Ace series.

All HPLC labeling reagents are high quality products, so you can make use of these products to achieve high quality analyses.

---- Products List by detection and functional groups ----

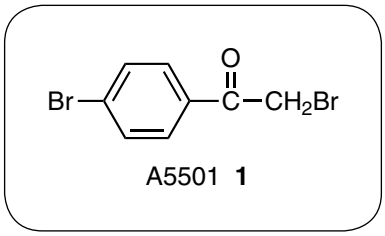
UV Detection

Fluorescene Detection

see also TCI product number list (p.102)

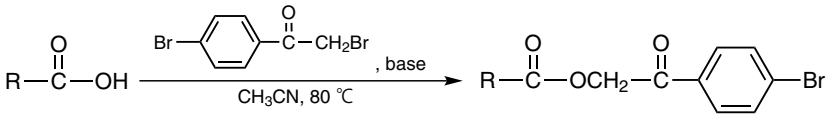
HPLC Labeling Reagent

for Carboxylic Acids



A5501 **1**

The compound **1** is an HPLC labeling reagent, which has a bromoacetyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Fatty acids][1, 2, 8, 9]

Dissolve a sample in methanol or water, and then neutralize the sample solution with methanol solution of KOH-crown ether. Evaporate to dryness under reduced pressure, and then you will see a generally almost white solid substance remaining (potassium salt of fatty acid). Next, add the HPLC labeling reagent **1** with acetonitrile solution* of 18-crown 6-ether to this white solid and further add acetonitrile for a volume up to 10 mL. Incubate the solution at 80 ºC for 15 min. Cool the resultant solution to room temperature and use it as an HPLC sample.

* Benzene can be used in the place of acetonitrile. The mixing ratio (molar ratio) for the HPLC labeling reagent **1** and 18-crown 6-ether should be 20 to 1 and 10 to 1 for the sample fatty acid concentrations at 0.5~20 mM and less than 0.5 mM, respectively. Use the excessive amount of the reagent **1**.

[Others]

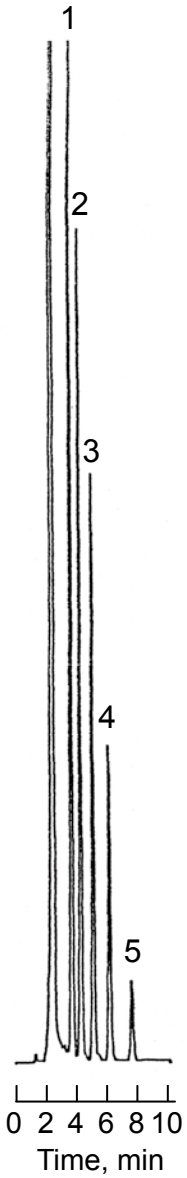
Dicarboxyl acids[2], synthetic prostaglandins[3], unsaturated fatty acids[4], alkyl methylphosphonate[5], ganglioside[6], betaine[7]

A5501	**4-Bromophenacyl Bromide**	5 g

References

1) H. D. Durst, *Anal. Chem.* **1975**, *47*, 1797.
2) E. Grushka, *J. Chromatogr.* **1975**, *112*, 673.
3) F. A. Fitzpatrick, *Anal. Chem.* **1976**, *48*, 499.
4) Y. Suzuki, T. Takeuchi, Nihon Gakujutsu Shinkoukai Tanka Suiso Kagaku Dai 116 Iinkai Gyoseki Houkoku **1976**, *29*, 152.
5) P. C. Bossle, J. J. Martin, E. W. Sarver, H. Z. Sommer, *J. Chromatogr.* **1983**, *267*, 209.
6) H. Nakabayashi, M. Iwamori, Y. Nagai, *J. Biochem.* **1984**, *96*, 977.
7) S. Konosu, A. Shinagawa, K. Yamaguchi, *Bull. Jpn. Soc. Sci. Fisher.* **1986**, *52*, 869.
8) M. Alberghina, A. Fiumara, L. Pavone, A. M. Giuffrida, *Neurochem. Res.* **1984**, *9*, 1719.
9) K. Kihara, S. Rokushika, H. Hatano, *Bunseki Kagaku* **1984**, *33*, 647.

Chromatogram of fatty acids as 4-bromophenacyl esters

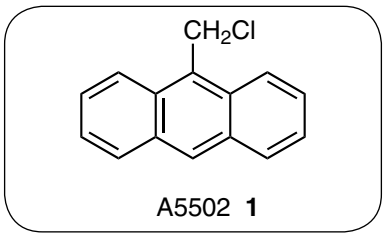


Column	: Kaseisorb LC C_1-60-5 4.6 mmI.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 80 / 20
Detector	: UV 254 nm
Flow Rate	: 1 mL / min

1. Lauric Acid
2. Myristic Acid
3. Palmitic Acid
4. Stearic Acid
5. Arachidic Acid

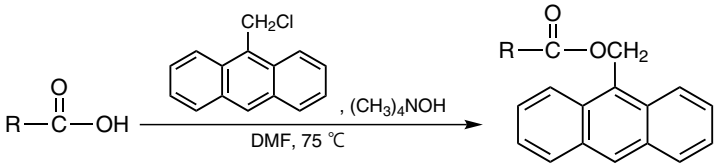
HPLC Labeling Reagent

for Carboxylic Acids



A5502 **1**

The compound **1**, an HPLC labeling reagent which has a chloromethyl group, easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection. Furthermore, it has a characteristic fluorescence based on an anthracene skeleton, thus carboxylic acids can be detected with the detection limit of 2 fmol by fluorescence detection analysis at the excitation and emission wavelengths of 365 nm and 412 nm, respectively.



Application example:

[Fatty acids][1)]

Dissolve 60 μg of a sample in 1 mL of DMF, and add 1 mL of tetramethylammonium hydroxide / DMF solution (1 x 10^{-3} M) and 1 mL of the labeling reagent **1** / cyclohexane solution (5 x 10^{-3} M). Close the cap of the reaction vessel and incubate the solution at 75 ºC for 30 min. Cool the resultant solution to room temperature and use it as an HPLC sample.

The detection limit = 0.1 pmol (UV detection: 254 nm)

The detection limit = 2 fmol (Fluorescence detection: λex 365 nm, λem 412 nm)

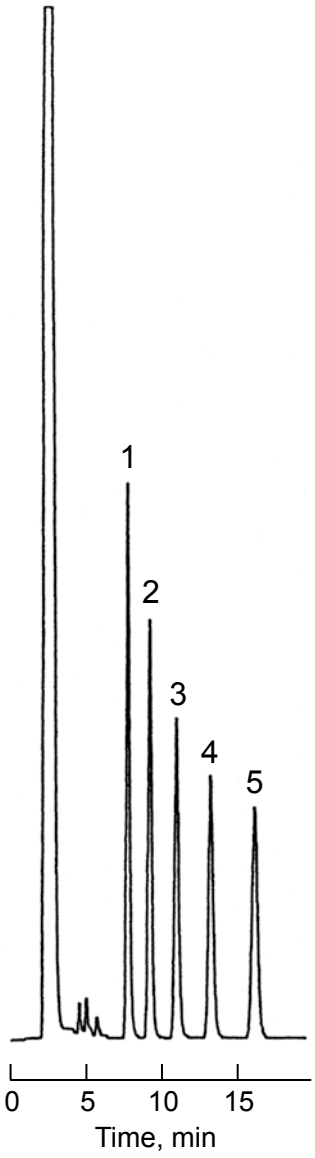
A5502	**9-Chloromethylanthracene**	1 g	5 g

Reference

1) W. D. Korte, *J. Chromatogr.* **1982**, *243*, 153.

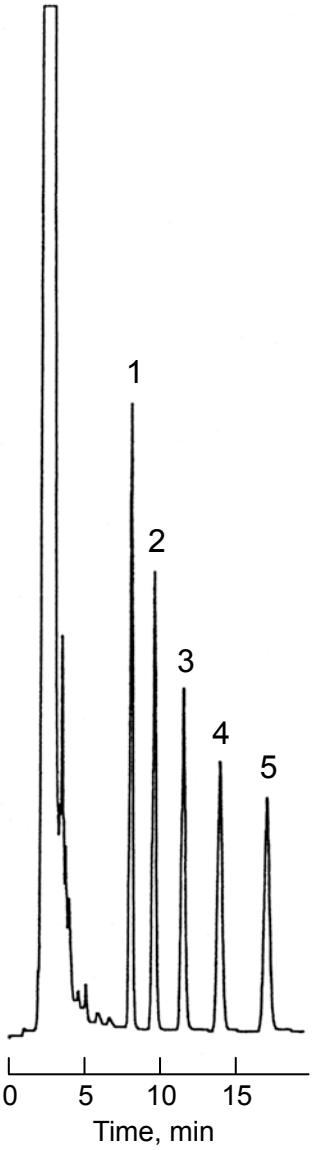
AZ-503

Chromatogram of fatty acids as 9-anthrylmethyl esters



Column : Kaseisorb LC C_8-60-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detector : Fluorescence λex 365 nm
λem 412 nm
Flow Rate : 1 mL / min

1. Lauric Acid
2. Tridecanoic Acid
3. Myristic Acid
4. Pentadecanoic Acid
5. Palmitic Acid

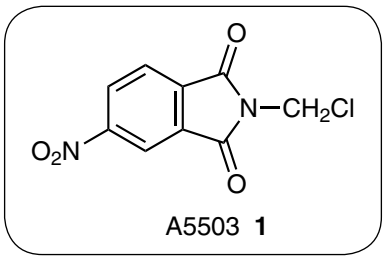


Column : Kaseisorb LC C_8-60-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Lauric Acid
2. Tridecanoic Acid
3. Myristic Acid
4. Pentadecanoic Acid
5. Palmitic Acid

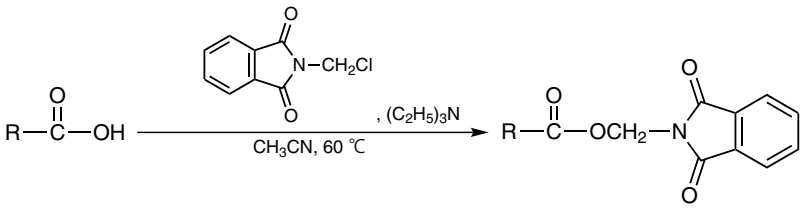
HPLC Labeling Reagent

for Carboxylic Acids



A5503 **1**

The compound **1** is an HPLC labeling reagent, which has a chloromethyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 230 nm.



Application example:

[Fatty acids][1, 2)]

Dissolve 3 mg of a sample in 1 mL of acetonitrile, and add 1 mL of the labeling reagent **1** / acetonitrile solution (11 mg / mL) and 1 mL of triethylamine / acetonitrile solution (5 mg / mL). Close the cap of the reaction vessel and incubate the solution at 60 ºC for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample. In the case of using alkali metal salts and crown ethers, the esterification reaction is completed in 15 min at 60 ºC. Cool the resultant solution to room temperature and use it as an HPLC sample.

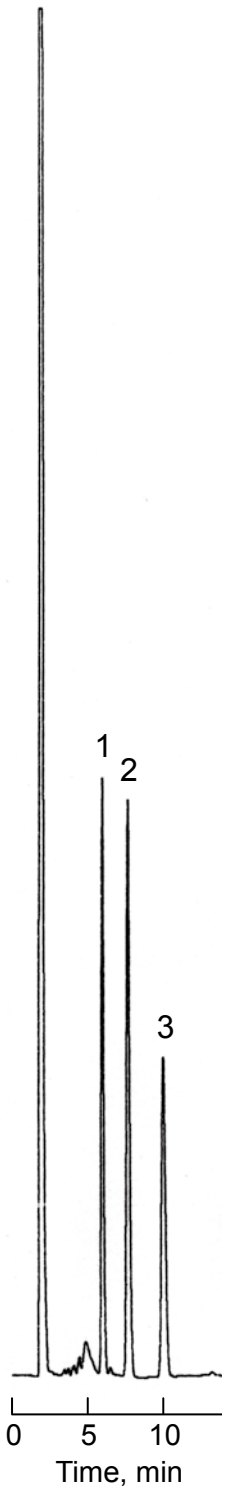
A5503	***N*-Chloromethyl-4-nitrophthalimide**	1 g	5 g

References

1) W. Lindner, *J. Chromatogr.* **1979**, *176*, 55.
2) W. Lindner, *J. Chromatogr.* **1980**, 198, 367.

AZ-504

Chromatogram of fatty acids as (4-nitrophthalimido)methyl esters

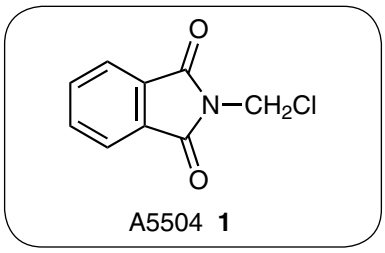


Column : Kaseisorb LC ODS-300-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 85 / 15
Detector : UV 230 nm
Flow Rate : 1 mL / min

1. Pentadecanoic Acid
2. Palmitic Acid
3. Margaric Acid

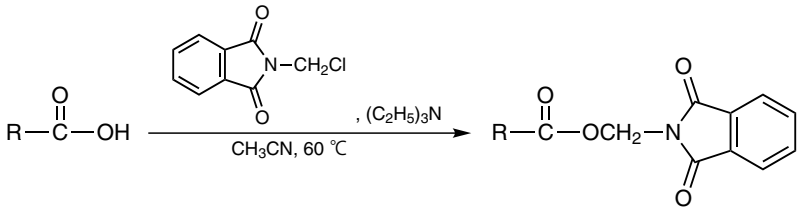
HPLC Labeling Reagent

for Carboxylic Acids



A5504 **1**

The compound **1** is an HPLC labeling reagent, which has a chloromethyl group and easily reacts with a carboxyl group to form an ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application example:

[Fatty acids] [1)]

Dissolve 3 mg of a sample in 1 mL of acetonitrile, and add 1 mL of the labeling reagent **1** / acetonitrile solution (10 mg / mL) and 1 mL of triethylamine / acetonitrile solution (5 mg / mL). Close the cap of the reaction vessel and incubate the solution at 60 ºC for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample. In the case of using alkali metal salts and crown ethers, the esterification reaction is completed in 5 min at 60 ºC. Cool the resultant solution to room temperature and use it as an HPLC sample.

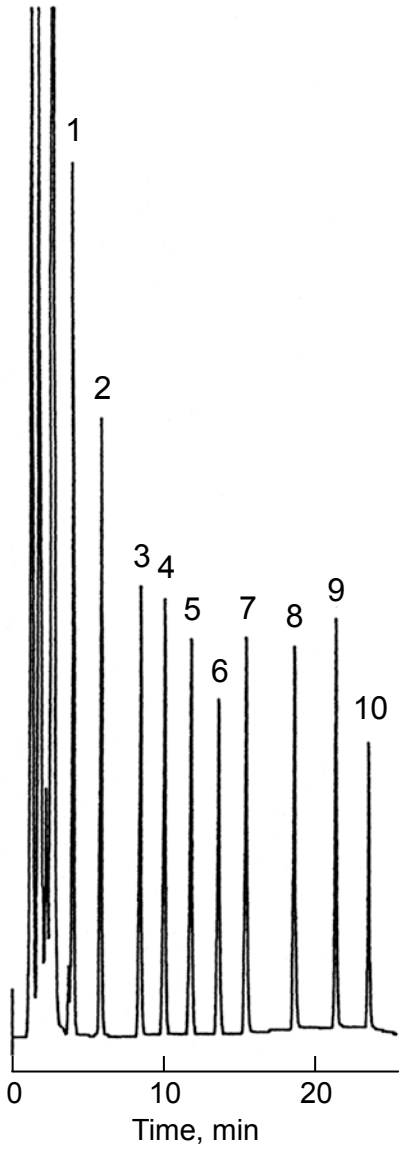
A5504	***N*-Chloromethylphthalimide**	5 g

Reference

1) W. Lindner, *J. Chromatogr.* **1979**, *176*, 55.

AZ-505

Chromatogram of fatty acids as phthalimidomethyl esters

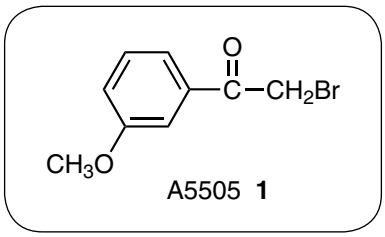


Column : Kaseisorb LC C_8-60-5
4.6 mmI.D.×150 mm
Mobile Phase: CH_3CN / H_2O =
70 / 30 → 100 / 0
20 min linear gradient
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Caproic Acid
2. Caprylic Acid
3. Capric Acid
4. Undecanoic Acid
5. Lauric Acid
6. Tridecanoic Acid
7. Myristic Acid
8. Palmitic Acid
9. Stearic Acid
10. Arachidic Acid

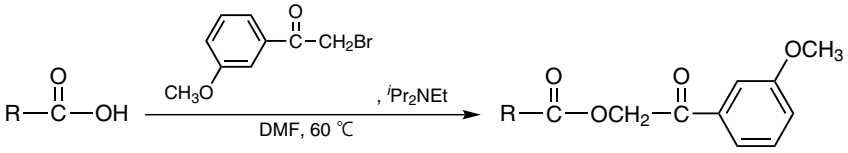
HPLC Labeling Reagent

for Carboxylic Acids



A5505 **1**

The compound **1** is an HPLC labeling reagent, which has a bromoacetyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application example:

[Fatty acids] [1~3)]

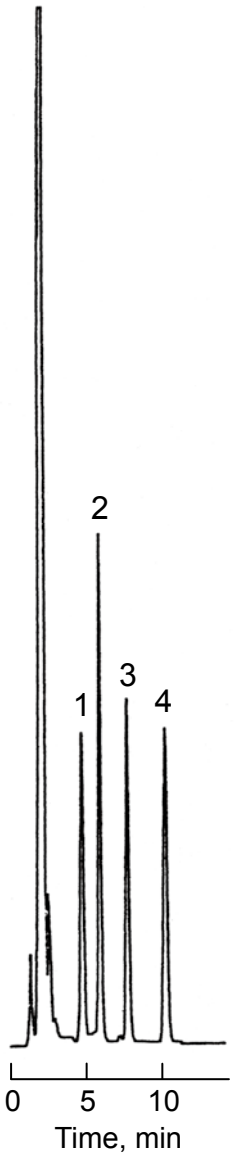
Dissolve 4 mg of a sample in 1 mL of *N*,*N*-dimethylformamide (DMF), and add the labeling reagent **1** (10 mg) in DMF (1 mL) and *N*,*N*-diisopropylethylamine (10 mg) in DMF (2 mL). Close the cap of the reaction vessel and incubate the solution at 60 ºC for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

A5505	**3'-Methoxyphenacyl Bromide**	5 g

References

1) R. A. Miller, N. E. Bussell, C. Ricketts, *J. Liquid Chromatogr.* **1978**, *1*, 291.
2) N. E. Bussell, R. A. Miller, *J. Liquid Chromatogr.* **1979**, *2,* 697.
3) N. E. Bussell, A. Gross, R. A. Miller, *J. Liquid Chromatogr.* **1979**, *2*, 1337.

Chromatogram of fatty acids as 3'-methoxyphenacyl esters

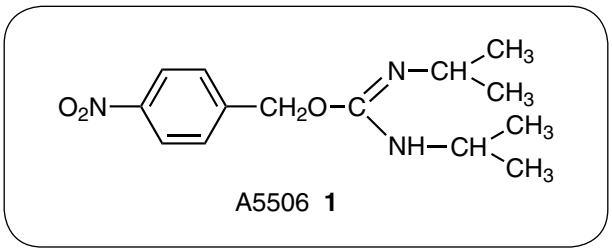


Column : Kaseisorb LC C_8-60-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

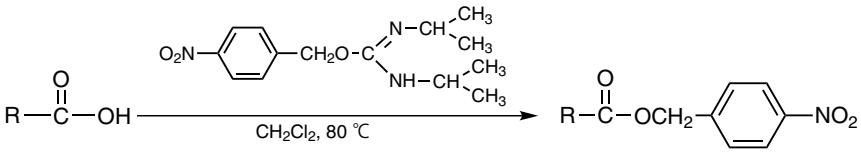
HPLC Labeling Reagent

for Carboxylic Acids



A5506 **1**

The compound **1** easily reacts with a carboxyl group to form the corresponding ester without using a catalyst or an activating agent. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application example:

[Fatty acids] [1)]

Dissolve 5 mg of a sample in CH_2Cl_2 (1 mL), and add the labeling reagent **1** (20 mg) in CH_2Cl_2 (2 mL). Close the cap of the reaction vessel and incubate the solution at 80 ºC for 2 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

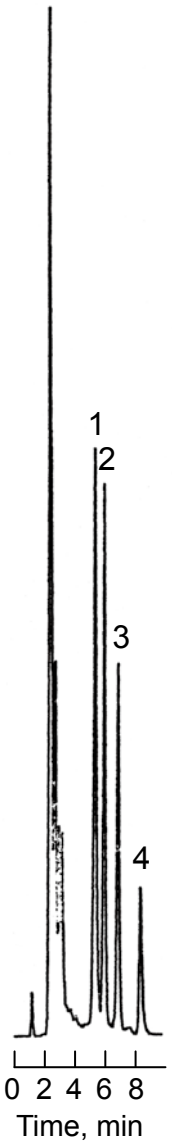
A5506 ***O*-(4-Nitrobenzyl)-*N,N*'-diisopropylisourea** 1 g

References

1) D. R. Knapp, S. Krueger, *Anal. Lett.* **1975**, *8*, 603.
2) B. Sbaikh, N. J. Pontzer, J. E. Molina, M. I. Kelsey, *Anal. Biochem.* **1978**, *85*, 47.
3) S. Okuyama, D. Uemura, Y. Hirata, *Bull. Chem. Soc. Jpn.* **1979**, *52*, 124.
4) R. Badoud, G. Pratz, *J. Chromatogr.* **1986**, *360*, 119.

AZ-507

Chromatogram of fatty acids as 4-nitrobenzyl esters

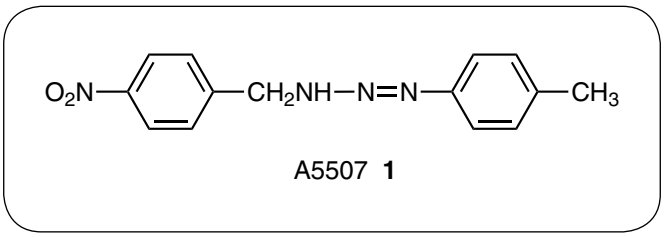


Column : Kaseisorb LC C_1-60-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 75 / 25
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

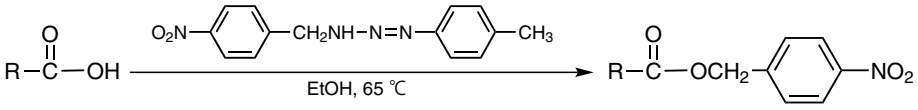
HPLC Labeling Reagent

for Carboxylic Acids



A5507 **1**

The compound **1** easily reacts with a carboxyl group to form the corresponding ester without using a catalyst or an activating agent. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Fatty acids] [1)]

Add 3 mL of ethanol and 50 mg of the labeling reagent **1** to 2~3 mg of a sample. Incubate the solution at 65 ºC for 1 h (Do not close the cap of the reaction vessel completely, because nitrogen gas is evolved during the reaction). Then, close the cap of the reaction vessel and cool the resultant solution to room temperature. Dilute with a suitable solvent and use it as an HPLC sample. If it is necessary to remove the unreacted labeling reagent and by-product, *p*-toluidine, evaporate the solvent at a low temperature under a nitrogen atmosphere after the derivatization reaction, and then dissolve the residue in 2~3 mL of ether and wash with diluted hydrochloric acid and water.

[Others]

HPLC of bile acids[2, 3)]

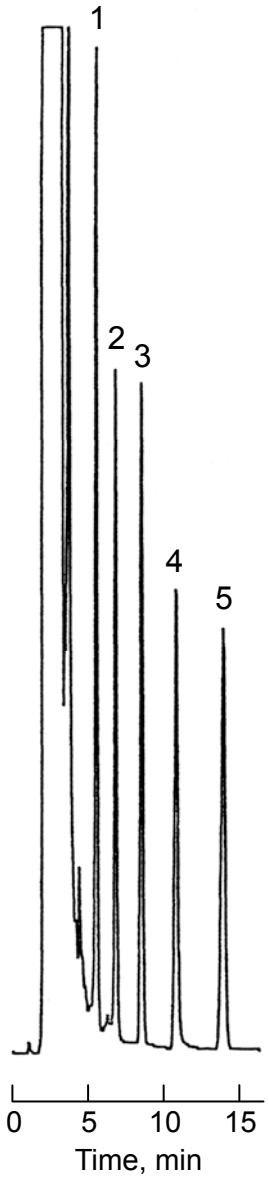
A5507 **1-(4-Nitrobenzyl)-3-*p*-tolyltriazene** 1 g

References

1) Regis Chemical Co., *Regis Lab. Notes*, August **1974**, No. 16
See also: I. R. Politzer, *Anal. Lett*. **1973**, *6*, 539.; *Org. Synth*. **1968**, *48*, 102.; *Org. Synth. Collect*. **1973**, *5*, 797.
2) S. Okuyama, D. Uemura, Y. Hirata, *Chem. Lett*. **1976**, 679.
3) B. Shaikh, N. J. Ponzer, J. E. Molina, M. I. Kelsey, *Anal. Biochem*. **1978**, *85*, 47.

AZ-508

Chromatogram of fatty acids as 4-nitrobenzyl esters

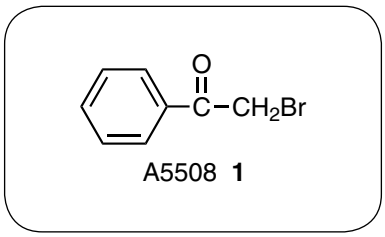


Column : Kaseisorb LC ODS-100-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Capric Acid
2. Undecanoic Acid
3. Lauric Acid
4. Tridecanoic Acid
5. Myristic Acid

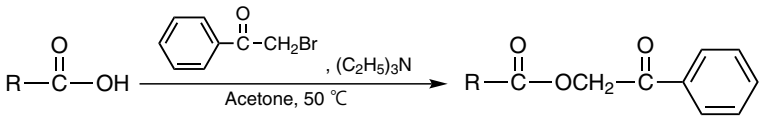
HPLC Labeling Reagent

for Carboxylic Acids



A5508 **1**

The compound **1** is an HPLC labeling reagent, which has a bromoacetyl group and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Fatty acids] [1)]

Mix *ca*. 100 μg of a sample, 10 μL of the labeling reagent **1** in acetone (12 mg / mL) and 10 μL of triethylamine in acetone (10 mg / mL), and incubate the solution at 50 ºC for 2 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

[Others]

Bile acids[2)], fatty acids[3)], carboxylic acids in wine[4)]

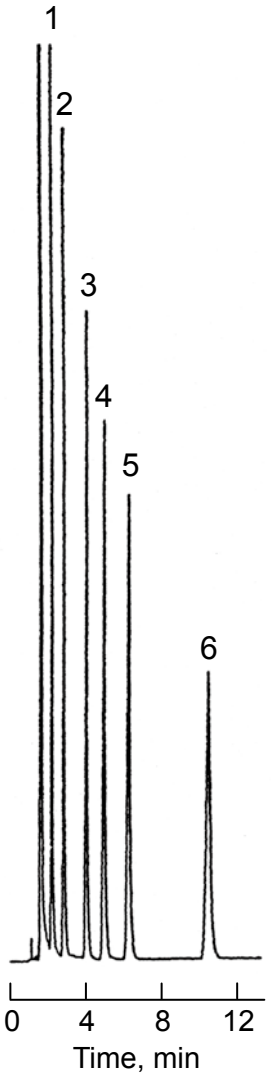
A5508 **Phenacyl Bromide** 5 g

References

1) R. F. Borch, *Anal. Chem.* **1975**, *47*, 2437.
2) F. Stellaard, *Anal. Biochem.* **1978**, *87*, 359.
3) K. Kihara, S. Rokushika, H. Hatano, *Bunseki Kagaku* **1984**, *33*, 647.
4) E. Mentasti, M. C. Gennaro, C. Sarzanini, C. Baiocchi, M. Savigliano, *J. Chromatogr.* **1985**, *322*, 177.

AZ-509

Chromatogram of fatty acids as phenacyl esters

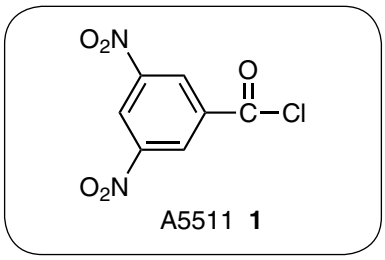


Column : Kaseisorb LC ODS-100-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 90 / 10
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Caproic Acid
2. Caprylic Acid
3. Capric Acid
4. Undecanoic Acid
5. Lauric Acid
6. Myristic Acid

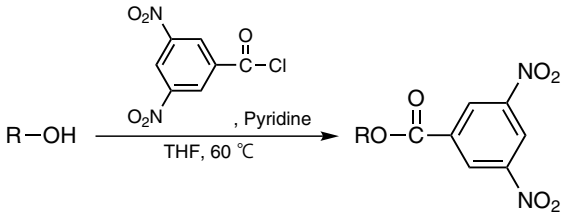
HPLC Labeling Reagent

for Alcohols and Amines



A5511 **1**

The compound **1** is an HPLC labeling reagent, which easily reacts with a hydroxyl group or an amino group to form the corresponding ester or amide, respectively. The resultant ester or amide is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Alcohols] [1)]

Dissolve 1~5 mg of a sample in 5 mL of THF, and add 40 mg of the labeling reagent **1** and a few drops of pyridine. Close the cap of the reaction vessel and incubate the solution at 60 ºC for 1 h. Cool the resultant solution to room temperature and use it as an HPLC sample.

Clean up before injection is recommended when pyridine or triethylamine is added to trap generated HCl. Generally, evaporate the solvent, extract with ether and wash the ether layer with diluted hydrochloric acid and water.

[Others]

Analysis of mono- and diethylene glycols in polyethylene glycol[2)], aliphatic alcohols[3)]

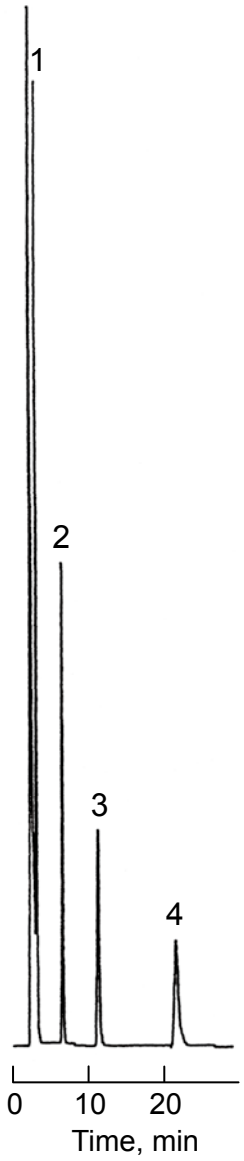
A5511 **3,5-Dinitrobenzoyl Chloride** 5 g

References

1) T. H. Jupille, *Am. Lab.* **1976**, *8*, 85.
2) M. A. Carey, H. E. Persinger, *J. Chromatogr. Sci.* **1972**, *10*, 537.
3) Y. Suzuki, N. Tsuchiya, *Bunseki Kagaku* **1981**, *30*, 240.
4) L. J. Elrod, L. B. White, S. G. Spanton, D. G. Stroz, P. J. Cugier, L. A. Luka, *Anal. Chem.* **1984**, *56*, 1786.

AZ-512

Chromatogram of alcohols as 3,5-dinitrobenzoic acid esters

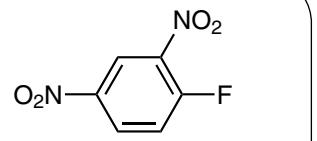


Column : Kaseisorb LC ODS-300-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 55 / 45
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Ethylene glycol
2. Ethanol
3. Propanol
4. Butanol

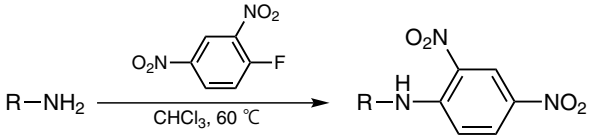
HPLC Labeling Reagent

for Amines



A5512 **1**

The compound **1** easily reacts with an amino group to form the corresponding 2,4-dinitrophenylamine derivative. The resultant derivative is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Amines]
A sample (free amine) 10 mg, chloroform 1 mL, and labeling regent **1** (10 eq. excess amount of the sample) are mixed, and incubated at 60 ºC for 1 h. After cooling to room temperature, use it as an HPLC sample. **1** is also used for derivatization of amino acids.[1,2)]

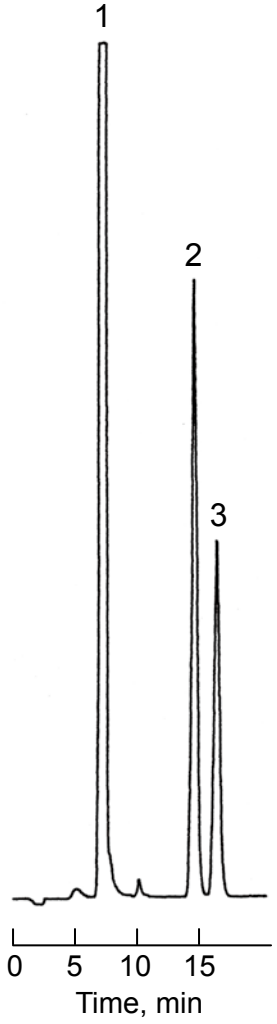
[Others]
Aminoglycosides[3)]

A5512	**2,4-Dinitrofluorobenzene**	5 g

References

1) Y. Suzuki, Program and Abstracts 6th Congress of Liquid Chromatography (October **1985**), 71.
2) S. A. Cockle, H. Kaplan, M. A. Hefford, N. M. Young, 1st High-Perform. Liq. Chromatogr. Proteins Pept., Proc. Int. Symp. **1983**, 103.
3) D. M. Barends, J. S. Blauw, C. W. Mijnsbergen, C. J. L. R. Govers, A. Hulshoff, *J. Chromatogr.* **1985**, *322*, 321.

Chromatogram of alkylamines as 2,4-dinitrophenyl derivatives

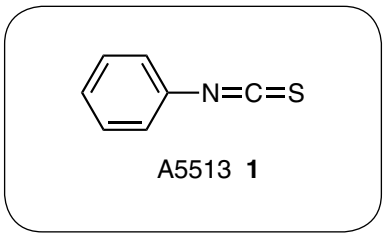


Column : Kaseisorb LC C_4-60-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 45 / 55
Detector : UV 254 nm
Flow Rate : 1 mL / min

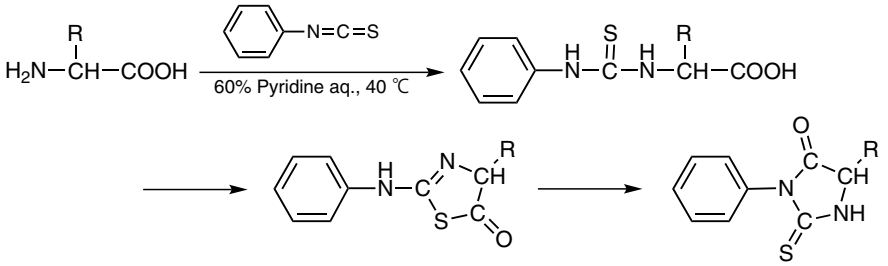
1. Labeling Reagent
2. Diethylamine
3. Propylamine

HPLC Labeling Reagent

for Amines



The compound **1** is an HPLC labeling reagent, which has an isothiocyano group, can easily react with an amino group to form the corresponding thiourea. The resultant thiourea can be also derivatized into a phenylthiohydantoin (PTH) derivative under acidic conditions. The PTH is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 269 nm for UV detection.



Application example:

[Amino acids, Peptides]

1.5 μmol of a sample is dissolved into 1 mL of 60% aqueous pyridine solution containing labeling reagent **1** (15 mg), and incubated at 40 ºC for 1 h. After cooling to room temperature, the reaction mixture is diluted with 1 mL of water, and excess amount of **1** is removed by extraction (benzene 2 mL x 4 times). The aqueous layer is evaporated, and dried in desiccator. To the residue, 1.5 mL of mixed solution (3 N HCl and 60% AcOH, 1 : 1) is added to hydrolyzed at 40 ºC for 30 min under a nitrogen atmosphere. After cooling to room temperature, the reaction mixture is diluted with 2 mL of water, and extracted with 2 mL of ethyl acetate, next 2 mL of benzene. The organic layers are combined to use it as an HPLC sample.

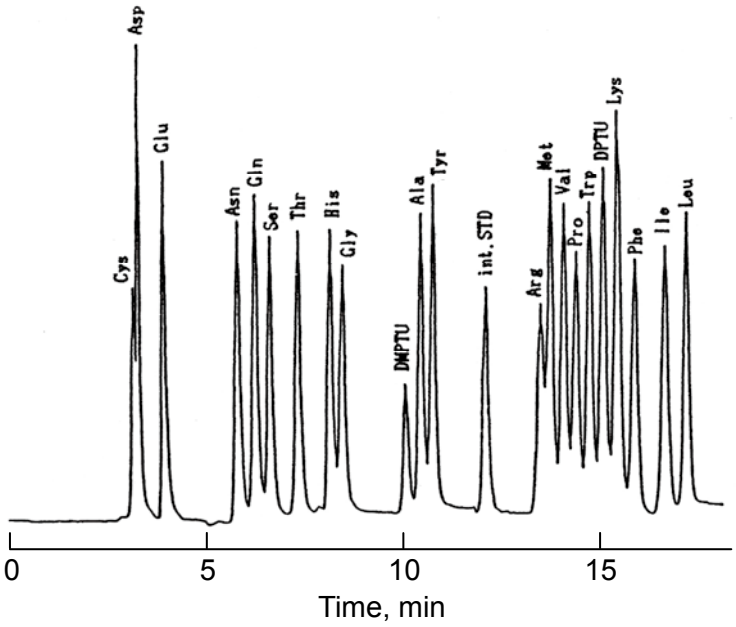
A5513	**Phenyl Isothiocyanate**	5 mL

References

1) P. Edman, G. Begg, *Eur. J. Biochem.* **1967**, *1*, 80.
2) V. M. Stepanov, *Anal. Biochem.* **1971**, *43*, 209.
3) G. Frank, W. Strubert, *Chromatographia* **1973**, *6*, 522.
4) A. P. Graffeo, *Anal. Lett.* **1973**, *6*, 505.
5) A. Hagg, K. Langern, *Chromatographia* **1974**, *7*, 659.
6) A. P. Graffeo, B. L. Karger, in *Instrumentation in Amino Acid Sequence Analysis*, ed. by R. N. Perham, Academic Press, London, New York, San Francisco, **1975**, p.111.
7) Z. Deyl, *J. Chromatogr.* **1976**, *127*, 91.
8) M. R. Downing, K. G. Mann, *Anal. Biochem.* **1976**, *74*, 298.
9) C. Z. Zimmerman, E. Appella, J. J. Pisano, *Anal. Biochem.* **1976**, *75*, 77.

AZ-514

Chromatogram of amino acids as PTH derivatives



Column
: Kaseisorb LC C_8-60-5
4.6 mmI.D.×300 mm
Mobile Phase
: A ;CH_3CN
: B ;40 mM CH_3COONa
: C ;H_2O
Detector : UV 269 nm
Flow Rate : 1 mL / min

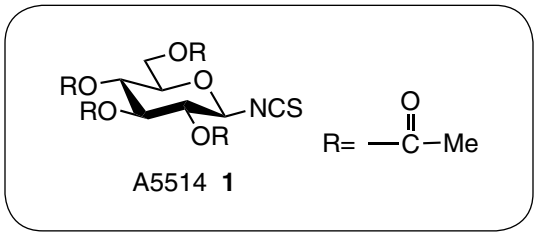
Time(min)	A(%)	B(%)	C(%)
0	36	20	44
3	42	20	38
4	45	25	30
5	50	30	20
9	52	30	18
12	65	5	30
13	36	20	44

(temp.40 ºC)

10) F. Trefz, O. J. Byrd, M. E. Blaskovics, W. Kochen, P. Lutz, *Clin. Chem. Acta* **1976**, *73*, 431.
11) F. G. Wing-Kin, E. Grushka, *J. Chromatogr.* **1977**, *142*, 299.
12) E. J. Kikta, E. Grushka, *J. Chromatogr.* **1977**, *135*, 367.
13) C. Z. Zimmerman, E. Appella, J. J. Pisano, *Anal. Biochem.* **1977**, *77*, 569.
14) W. T. Butler, J. E. Finch, E. J. Miller, *J. Biol. Chem.* **1977**, *252*, 639.
15) M. N. Margolies, A. Brauer, *J. Chromatogr.* **1978**, *148*, 447.
16) M. Abrahamsson, K. Gröningsson, S. Castensson, *J. Chromatogr.* **1978**, *154*, 313.
17) J. Elion, M. Downing, K. Mann, *J. Chromatogr.* **1978**, *155*, 436 .
18) A. S. Bhown, J. E. Mole, W. L. Holloway, C. Bernett, *J. Chromatogr.* **1978**, *156*, 35.
19) R. L. Heinrikson, S. C. Meredith, *Anal. Biochem.* **1984**, *136*, 65.
20) J. J. L'Italien, S. B. H. Kent, *J. Chromatogr.* **1984**, *283*, 149.
21) R. R. Granberg, *LC, Liq. Chromatogr. HPLC Mag.* **1984**, *2*, 776.
22) B. A. Bidlingmeyer, S. A. Cohen, T. L. Tarvin, *J. Chromatogr.* **1984**, *336*, 93.
23) D. L. Christie, R. M. Hill, K. Isakow, P. M. Barling, *Anal. Biochem.* **1986**, *154*, 92.
24) S. A. Cohen, B. A. Bidlingmeyer, T. L. Tarvin, *Nature (London)* **1986**, *320*, 769.
25) L. E. Lavi, J. S. Holcenberg, D. E. Cole, J. Jolivent, *J. Chromatogr.* **1986**, *377*, 155.
26) D. Lanneluc-Sanson, C. T. Phan, R. L. Granger, *Anal. Biochem.* **1986**, *155*, 322.
27) V. Semensi, M. Sugumaran, *LC-GC* **1986**, *4*, 1108.
28) A. Lilova, T. Kleinschmidt, P. Nedkov, G. Braunitzer, *Biol. Chem. Hoppe-Seyler* **1986**, *367*, 1055.

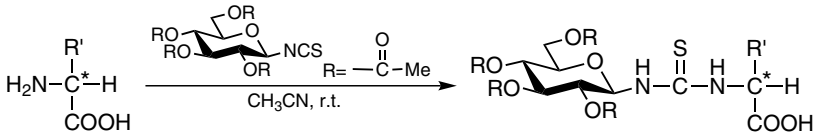
HPLC Labeling Reagent

for Amines



A5514 **1**

The compound **1** is an HPLC labeling reagent for optical purity determination, which has a glycomoiety and an isothiocyano group, and easily reacts with an amino group to form the corresponding thiourea. The resultant thiourea is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection. Furthermore, **1** reacts with a racemic amine to generate diastereomers, which can be efficiently separated by reversed phase HPLC.



Application examples:

[Amino acids] [1)]

5 mg of an amino acid is dissolved in 50% (V/V) aqueous acetonitrile containing 0.4% (W/V) triethylamine in order to give a final volume of 10 mL. To 50 μL of this solution 50 μL of 0.2% (W/V) labeling reagent **1** in acetonitrile are added. The resulting mixture is shaken at room temperature for 30 min and used as an HPLC sample.

[Others]

Propranolol[2)], trimetoquinol[3)]

A5514	**2,3,4,6-Tetra-*O*-acetyl-β-D-glucopyranosyl Isothiocyanate**	100 mg	1 g

References

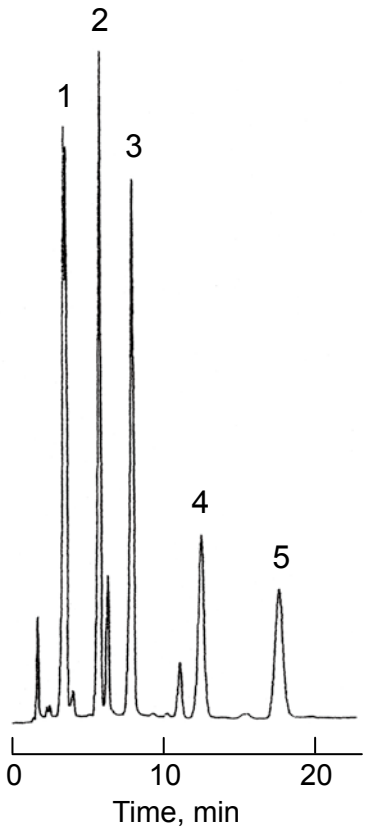
1) T. Kinoshita, Y. Kasahara, N. Nimura, *J. Chromatogr.* **1981**, *210*, 77.

2) A. J. Sedman, J. Gal, *J. Chromatogr.* **1983**, *278*, 199.

3) H. Nishi, N. Fujimura, H. Yamaguchi, T. Fukuyama, *J. Chromatogr.* **1991**, *539*, 71.

AZ-515

Chromatogram of thiourea derivatives formed from amino acids with GITC

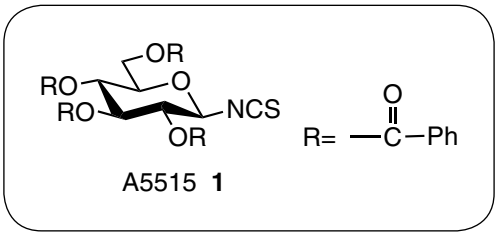


Column : Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm
Mobile Phase : 10 mM Phosphate buffer / Methanol = 45 / 55 (pH 3.0)
Detector : UV 254 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min

1. Aspartic Acid
2. L-Valine
3. D-Valine
4. L-Tryptophan
5. D-Tryptophan

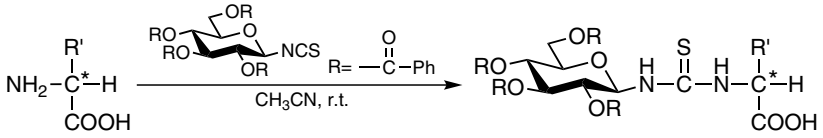
HPLC Labeling Reagent

for Amines



A5515 **1**

The compound **1** is an HPLC labeling reagent for optical purity determination, which has a glycomoiety and an isothiocyano group, and easily reacts with an amino group to form the corresponding thiourea. The resultant thiourea is stable and can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection. Furthermore, **1** reacts with a racemic amine to generate diastereomers, which can be efficiently separated by reversed phase HPLC.



Application example:

[Amino acids][1)]

5 mg of an amino acid is dissolved in 50% (V/V) aqueous acetonitrile containing 0.55% (V/V) triethylamine in order to give a final volume of 10 mL. To 50 μL of this solution 50 μL of 0.66% (W/V) labeling reagent **1** in acetonitrile are added. The resulting mixture is shaken at room temperature for 30 min, then 10 μL of 0.26% (V/V) ethanolamine in acetonitrile are added and shaken for another 10 min. The mixture is diluted with acetonitrile to a final volume of 1 mL and used as an HPLC sample.

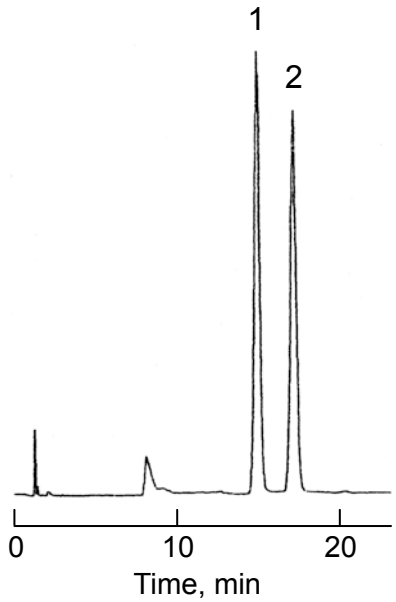
A5515	**2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl Isothiocyanate**	100 mg	1 g

Reference

1) M. Lobell, M. P. Schneider, *J. Chromatogr.* **1993**, *633*, 287.

AZ-516

Chromatogram of thiourea derivatives formed from amino acids with BGIT

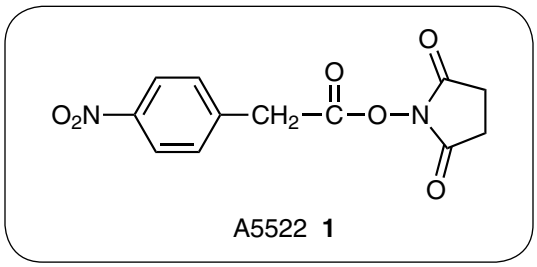


Column : Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm
Mobile Phase : 10 mM Phosphate buffer / CH_3CN = 35 / 65 (pH 3.0)
Detector : UV 254 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min

1. L-Phenylalanine
2. D-Phenylalanine

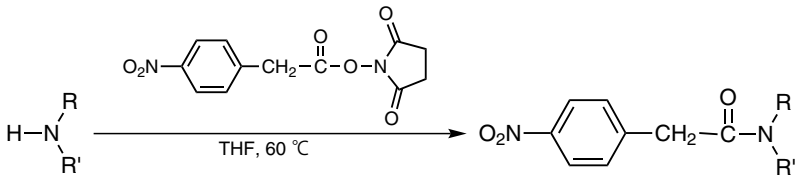
HPLC Labeling Reagent

for Amines



A5522 **1**

The compound **1** is an HPLC labeling reagent, which has a succinimidyl group, which can easily react with an amino group to form the corresponding amide derivative. The resultant amide is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Alkylamines]

1~5 mg of a sample (free amine), 5 mL of THF, and 50 mg of labeling reagent **1** are mixed, and incubated at 60ºC for 1 h. After cooling to room temperature, use it as an HPLC sample. If it is necessary to remove the unreacted labeling reagent and by-product, *N*-hydroxysuccinimide, evaporate the solvent at a low temperature under a nitrogen atmosphere. Dissolve the residue in 2~3 mL of ether and wash with aqueous $NaHCO_3$ and water.

[Others]

Drugs (amphetamine, methamphetamine)[1)]

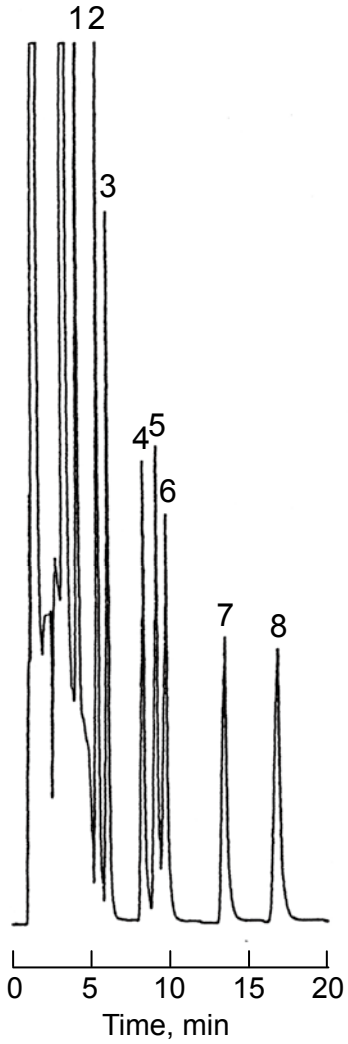
A5522	***N*-Succinimidyl 4-Nitrophenylacetate**	1 g

Reference

1) T. H. Jupille, *Am. Lab.* **1976**, *8*, 85.

AZ-523

Chromatogram of alkylamines as 4-nitrophenylacetamides

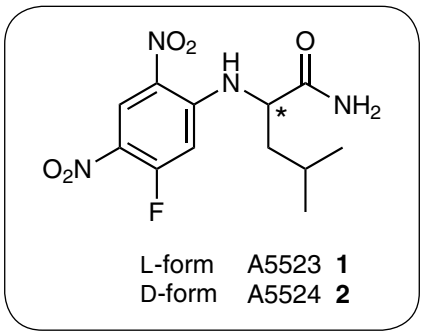


Column : Kaseisorb LC ODS-100-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3OH / H_2O = 60 / 40
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Propylamine
2. Diethylamine
3. Butylamine
4. Ethylpropylamine
5. Isoamylamine
6. Amylamine
7. Dipropylamine
8. Hexylamine

HPLC Labeling Reagent

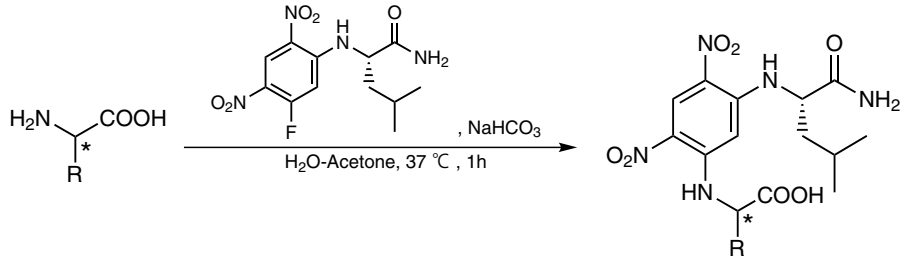
for Amines



L-form A5523 **1**
D-form A5524 **2**

The compounds **1** and **2** are HPLC labeling reagents for optical purity determination, and can easily react with amino groups. **1** or **2** reacts with a racemic amino acid to generate diastereomers, which can be efficiently separated by reversed phase HPLC. The absolute configuration of amino acids also can be non-empirically determined with use of **1** and **2**. Furthermore, high sensitive analyses can easily be accomplished using LC-MS. [The detection limit: 5 pmol (ESI LC-MS)]

Example : L-form



Application example:

[Amino acids] [2)]

To 50 μL of a 50 mM aqueous solution of amino acids are added 20 μL of 1 M $NaHCO_3$ and then 100 μL of 1% labeling reagent **1** or **2** in acetone. The solution is incubated at 37 ºC for 1 h. Reactions are quenched by addition of 20 μL of 1 N HCl. Samples are diluted with 810 μL of acetonitrile, and 1 μL of this solution is analyzed by LC-MS.

A5523	***N*$^{\alpha}$-(5-Fluoro-2,4-dinitrophenyl)-L-leucinamide**		100 mg
A5524	***N*$^{\alpha}$-(5-Fluoro-2,4-dinitrophenyl)-D-leucinamide**	100 mg	1 g

References

1) K. Fujii, Y. Ikai, H. Oka, M. Suzuki, K.-I. Harada, *Anal. Chem.* **1997**, *69*, 5146.
2) K. Fujii, Y. Ikai, T. Mayumi, H. Oka, M. Suzuki, K.-I. Harada, *Anal. Chem.* **1997**, *69*, 3346.

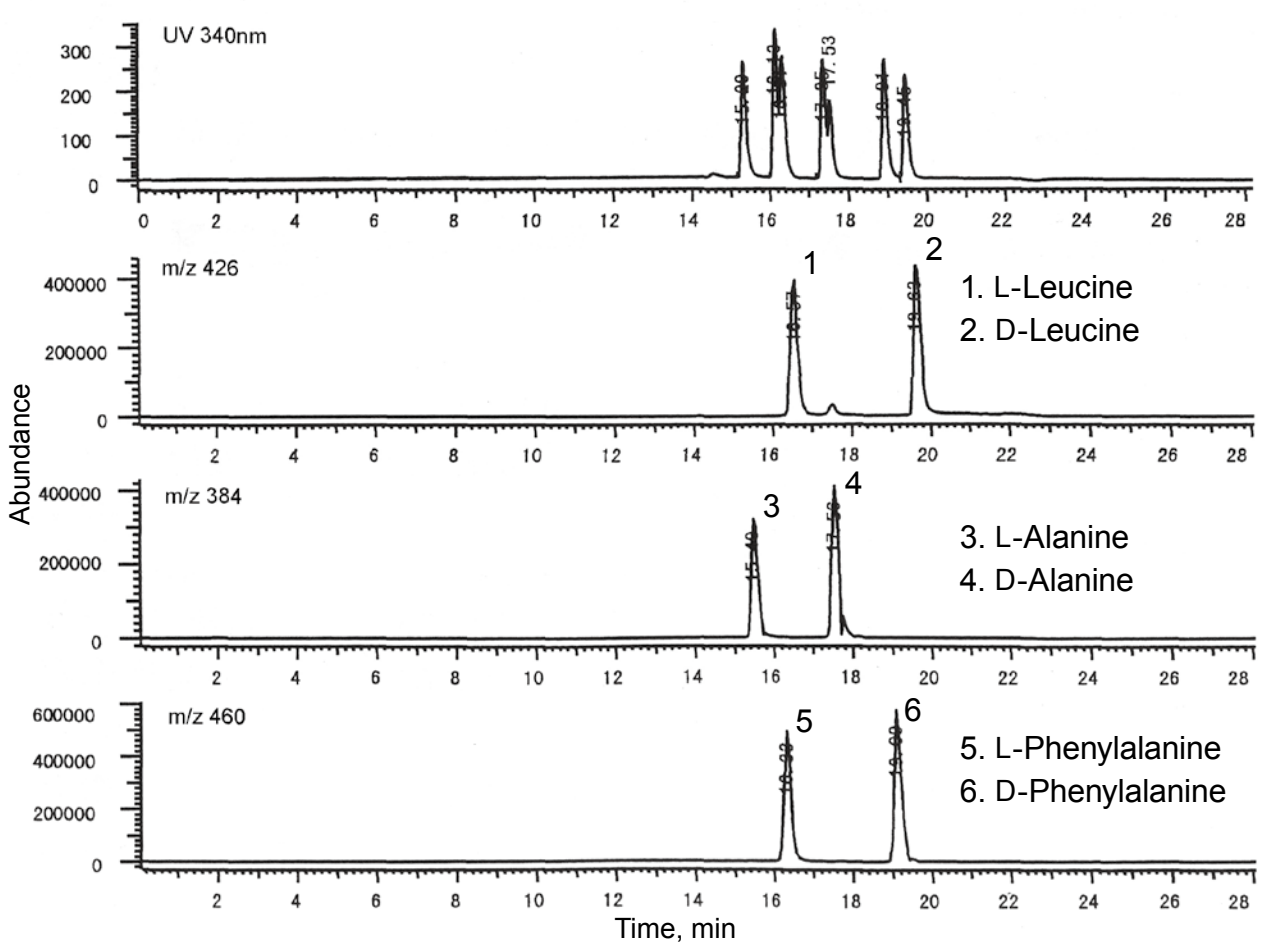
AZ-524

Chromatogram of amino acids as L-FDLA derivatives

Column	: Kaseisorb LC ODS 2000 2.0 mmI.D.×150 mm
Mobile Phase	: A- 20 mM Ammonium Acetate (pH 4) B- Methanol

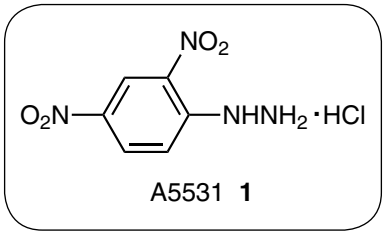
Time(min)	A(%)	B(%)
0	90	10
4	50	50
20	0	100
23	0	100

Temperature	: 40 ºC
Flow Rate	: 0.2 mL / min
Instrument	: Hitachi M-8000 LC/3DQ MS
Ionization Method	: ESI-AD



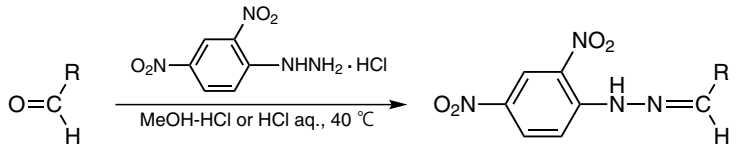
HPLC Labeling Reagent

for Carbonyl Compounds



A5531 **1**

The compound **1** is an HPLC labeling reagent, which has a hydrazino group and easily reacts with a carbonyl group to form the corresponding hydrazones. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application examples:

[Aldehydes]

1 mg of a sample, 1 mg of the labeling reagent **1**, 1 mL of methanol, and 0.5 mL of 1 N HCl are mixed. Close the cap of the reaction vessel and incubate the mixture at 40 ºC for 10 min. After cooling to room temperature, use it as the HPLC sample solution.

[Keto acids] [1,2)]

A sample is dissolved in 1 mL of diluted HCl solution containing labeling reagent **1** (500 μmol / 2 N HCl 100 mL). Incubate the mixture at 30 ºC for 30 min(The reactions are completed in 5 min and 20 min for ketomonocarboxylic acids and ketodicarboxylic acids, respectively.). It is preferable to add over 4 eq. amount of the labeling reagent, and resultant hydrazones can be extracted with ethyl acetate.

[Urine, 17-Ketosteroids in blood plasma] [3,4)]

A sample is dissolved into methanol, and acidified with 3~4 drops of conc. HCl. Excess amount of 0.2% labeling reagent **1** in methanol is added. Incubate the mixture at 50 ºC for 5 min.

[Others]

Aliphatic carbonyl compounds[5,6], aliphatic aldehydes[7~9]

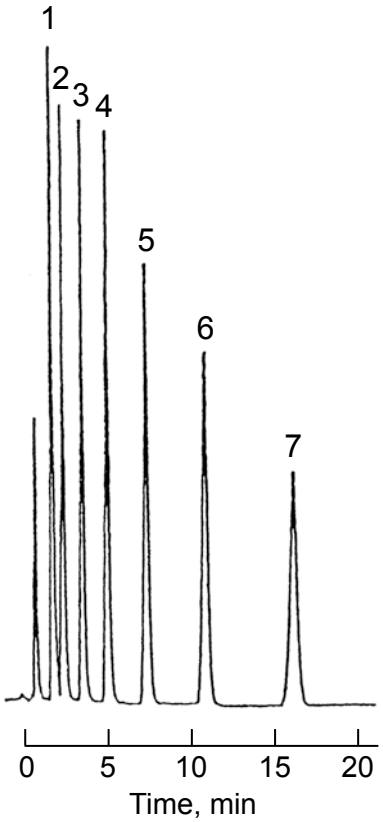
A5531 **2,4-Dinitrophenylhydrazine Hydrochloride** 5 g

References

1) H. Katsuki, *Anal. Biochem.* **1968**, *24*, 112.
2) N. Ariga, *Anal. Biochem.* **1972**, *49*, 436.
3) F. A. Fitzpatrick, *Anal. Chem.* **1972**, *44*, 2211.
4) R. A. Henry, *J. Chromatogr. Sci.* **1971**, *9*, 513.

AZ-532

Chromatogram of aldehydes as 2,4-dinitrophenylhydrazone



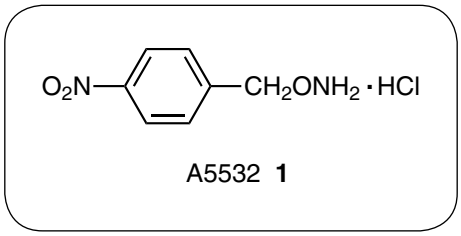
Column : Kaseisorb LC ODS-60-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 70 / 30
Detector : UV 254 nm
Flow Rate : 1 mL / min

1. Formaline
2. Acetaldehyde
3. Propionaldehyde
4. Butyraldehyde
5. Valeraldehyde
6. Capronaldehyde
7. Heptylaldehyde

5) M. A. Carey, H. E. Persinger, *J. Chromatogr. Sci.* **1972**, *10*, 537.
6) L. J. Papa, L. P. Turner, *J. Chromatogr. Sci.* **1972**, *10*, 747.
7) Y. Suzuki, H. Maruyama, *Bunseki Kagaku* **1979,** *28*, 671.
8) Y. Suzuki, H. Maruyama, *Bunseki Kagaku* **1985**, *34*, 717.
9) M. Uehori, K. Kuwata, Y. Yamazaki, *Annual report of Environmental Pollution Control Center Osaka Prefecture* **1982**, *5*, 27.

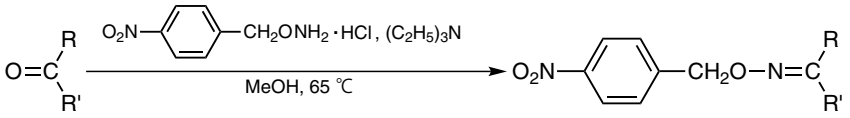
HPLC Labeling Reagent

for Carbonyl Compounds



A5532 **1**

The compound **1** is an HPLC labeling reagent, which has a hydroxylamino moiety, can easily react with a carbonyl group to form the corresponding oxime. The resultant oxime is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by measuring at 254 nm, an absorption wavelength that is generally used for UV detection.



Application example:

[Aldehydes] [1)]

1~5 mg of a sample, 4 mL of methanol, 2 drops of triethylamine, and 40 mg of the labeling reagent **1** are mixed. Close the cap of the reaction vessel and incubate the mixture at 65 °C for 1 h. After cooling to room temperature, use it as the HPLC sample solution. If it is necessary to remove the unreacted labeling reagent and triethylamine, evaporate the solvent at a low temperature under a nitrogen atmosphere. Dissolve the residue in 2~3 mL of ether and wash with diluted HCl and water.

A5532	***O*-4-Nitrobenzylhydroxylamine Hydrochloride**	1 g	5 g

Reference

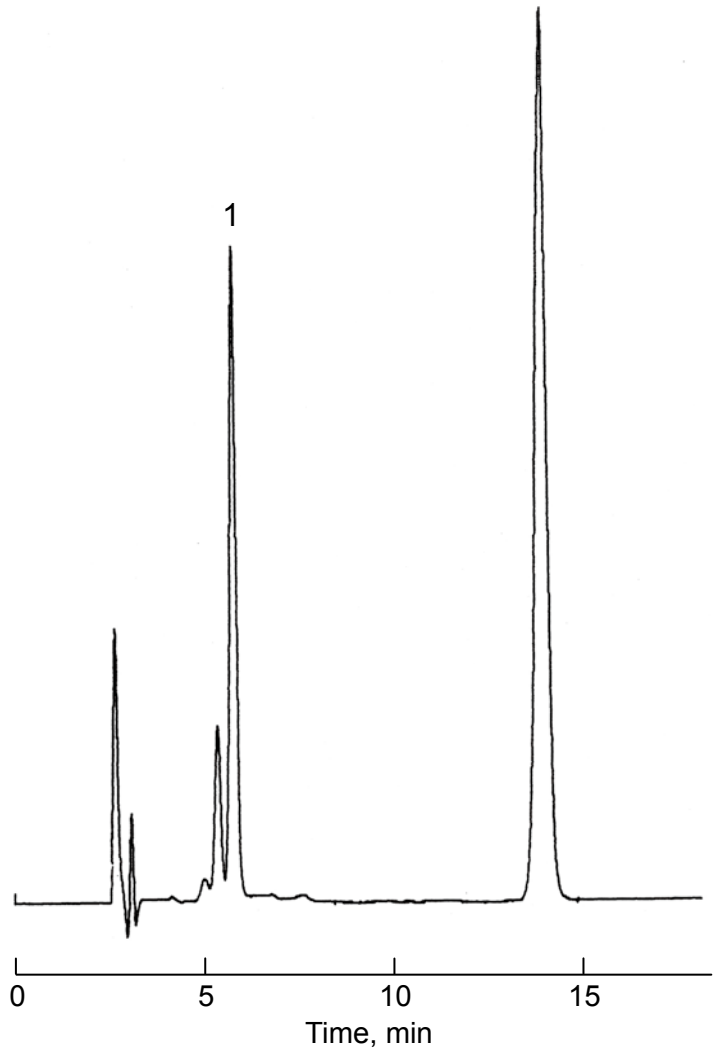
1) T. H. Jupille, *Am. Lab.* **1976**, *8*, 85.

AZ-533

Chromatogram of glucose as dabsyl hydrazone

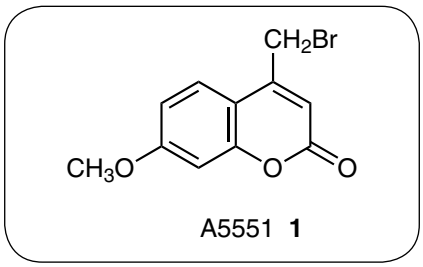
Column : Kaseisorb LC ODS-300-5
4.6 mmI.D.×250 mm
Mobile Phase : CH_3CN / H_2O = 35 / 65
Detector : Visible 425nm
Flow Rate : 1 mL / min

1. Glucose



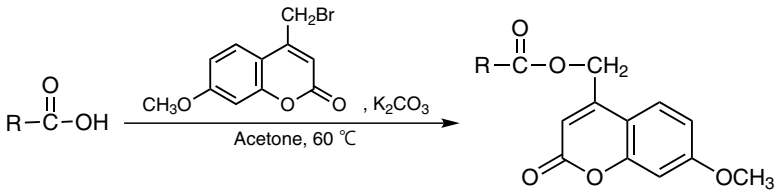
HPLC Labeling Reagent

for Carboxylic Acids



A5551 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a bromomethyl group, can easily react with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable enough to reach the detector without any decomposition under reversed phase HPLC. Furthermore, it has a characteristic fluorescence based on a coumarin skeleton, thus an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 328 nm and 380 nm, respectively.



Application examples:

[Fatty acids] [1)]

0.05 g of the labeling reagent **1** and 0.5 g of K_2CO_3 powder is added to a acetone solution (5 mL) of a sample (0.01 g), and incubate at 60 ºC for 1 h. After cooling to room temperature, use it as the HPLC sample solution.

[Others]

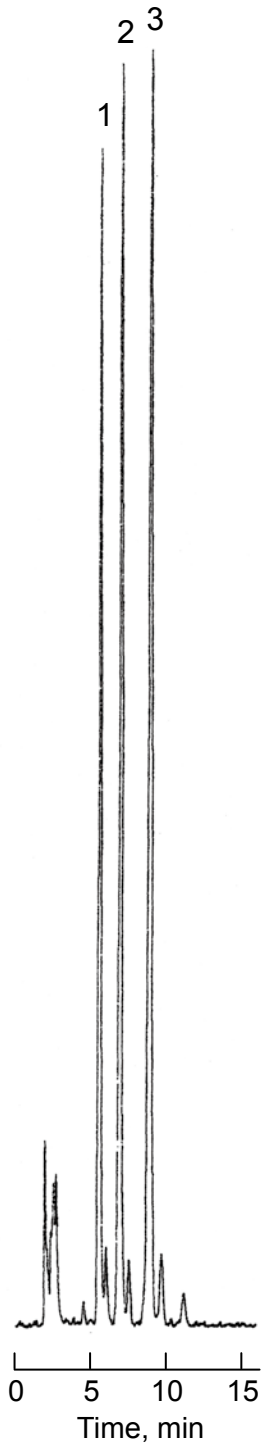
Carboxylic acids[2)] aliphatic acids[3)] dicarboxylic acids[4)] prostagrandins[5)] bile acids[6)] barbitals[7)]

A5551	**Br-Mmc (=4-Bromomethyl-7-methoxycoumarin)**	1 g	5 g

References

1) W. Dünges, *Anal. Chem.* **1977**, *49*, 442.
2) S. Lam, E. Grushka, *J. Chromatogr.* **1978**, *158*, 207.
3) S. G. Zelenski, J. W. Huber, *Chromatographia* **1978**, *11*, 645.
4) E. Grushka, *Anal. Chem.* **1978**, *50*, 1398.
5) J. Turk, *Prostaglandins* **1978**, *16*, 291.
6) S. Okuyama, *Chem. Lett.* **1979**, 461.
7) W. Dünges, N. Seiler, *J. Chromatogr.* **1978**, *145*, 483.
8) M. L. Grayeski, K. D. Joseph, *Anal. Chem.* **1987**, *59*, 1203.

Chromatogram of fatty acids as methoxycoumarinylmethyl esters

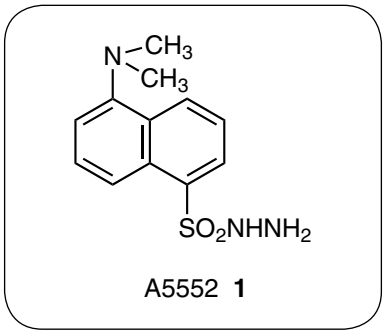


Column : Kaseisorb LC ODS-100-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 85 / 15
Detector : Fluorescence λex 328 nm
λem 380 nm
Flow Rate : 1 mL / min

1. Pelargonic Acid
2. Capric Acid
3. Undecanoic Acid

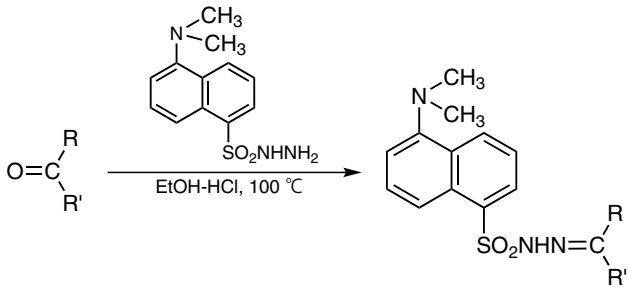
HPLC Labeling Reagent

for Carbonyl Compounds



A5552 **1**

The compound **1** is an HPLC fluorescence labeling reagent, and can easily react with a carbonyl group to form the corresponding hydrazone. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 340 nm and 525 nm, respectively.



Application examples:

[Ketosteroids] [1~4)]

A dried sample, 0.2 mL of an alcoholic hydrochloric acid (conc. HCl 0.65 mL / ethanol 1 L), and 0.2 mL of the labeling reagent **1** in alcohol (2 mg / mL) are mixed, and heated on a water bath for 10 min. 0.2 mL of alcohol containing sodium pyruvate (5 mg / mL) is added to decompose the excess labeling reagent. The reaction mixture is allowed to stand at room temperature for 15 min, ether (6 mL) and 0.5 N NaOH (3 mL) are added and shaken. After an extraction procedure, the solvent is evaporated, chloroform (0.2~0.5 mL) is added to the residue, and use as the HPLC sample.

[Others]

Hydrocortisone in body fluid[3,4)], reducing sugars, steroids in serum and urine[5)]

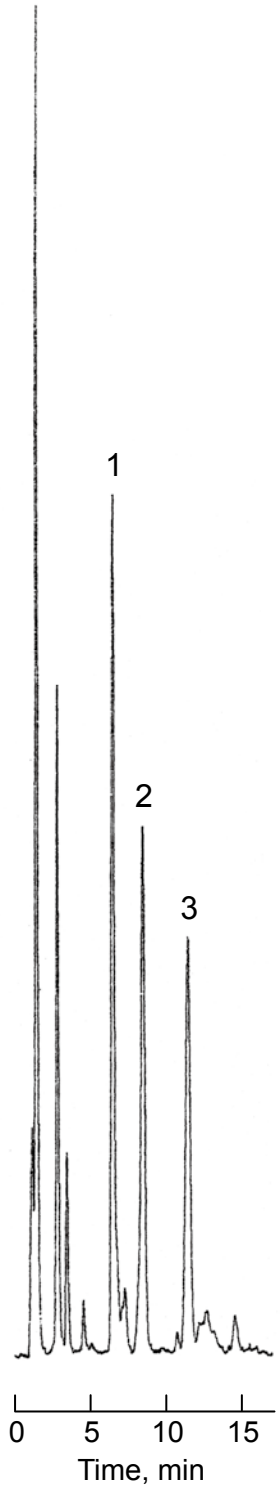
A5552	**Dansyl Hydrazine**	1 g	5 g

References

1) R. Chayen, R. Dvir, S. Gould, A. Harell, *Anal. Biochem.* **1971**, *42*, 283.
2) C. Apter, R. Chayen, S. Gould, A. Harell, *Clin. Chim. Acta* **1972**, *42*, 115.
3) T. Kawasaki, M. Maeda, A. Tsuji, *J. Chromatogr.* **1979**, *163*, 143.
4) T. J. Goehl, G. M. Sundaresan, V. K. Prasad, *J. Pharm. Sci.* **1979**, *68*, 1374.
5) T. Kawasaki, M. Maeda, A. Tsuji, *J. Chromatogr.* **1981**, *226*, 1.

AZ-553

Chromatogram of aldehydes as dansyl hydrazones

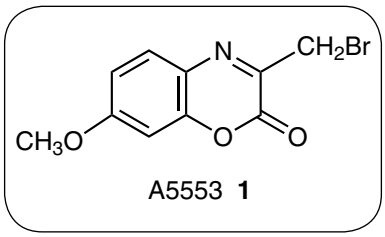


Column	: Kaseisorb LC ODS-100-5 4.6 mmI.D.×150 mm		
Mobile Phase	: CH_3CN / H_2O = 65 / 35		
Detector	: Fluorescence	λex	340 nm
		λem	525 nm
Flow Rate	: 1 mL / min		

1. Valeraldehyde
2. Capronaldehyde
3. Enanthic Aldehyde

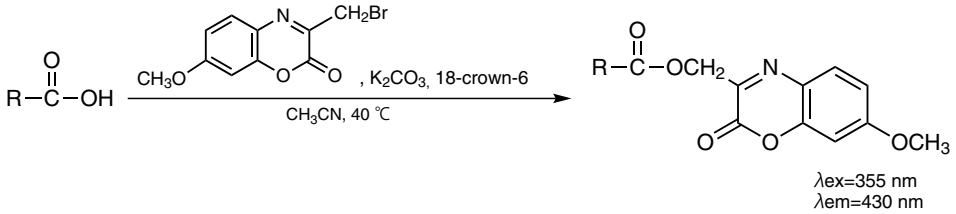
HPLC Labeling Reagent

for Carboxylic Acids



A5553 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a bromomethyl group, can easily react with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 355 nm and 430 nm, respectively.



Application example:

[Fatty acids] [1)]

A solution of the labeling reagent **1** (0.1 mL, 1.0 mM acetonitrile solution) is added to a solution of a fatty acid (0.5 mL, 0.2~10 nmol in acetonitrile). To this solution, a saturated K_2CO_3 / acetonitrile solution (0.5 mL) containing 18-crown 6-ether (5.7 mM) is added, and incubate at 40 ºC for 30 min. After cooling to room temperature, use it as the HPLC sample solution.

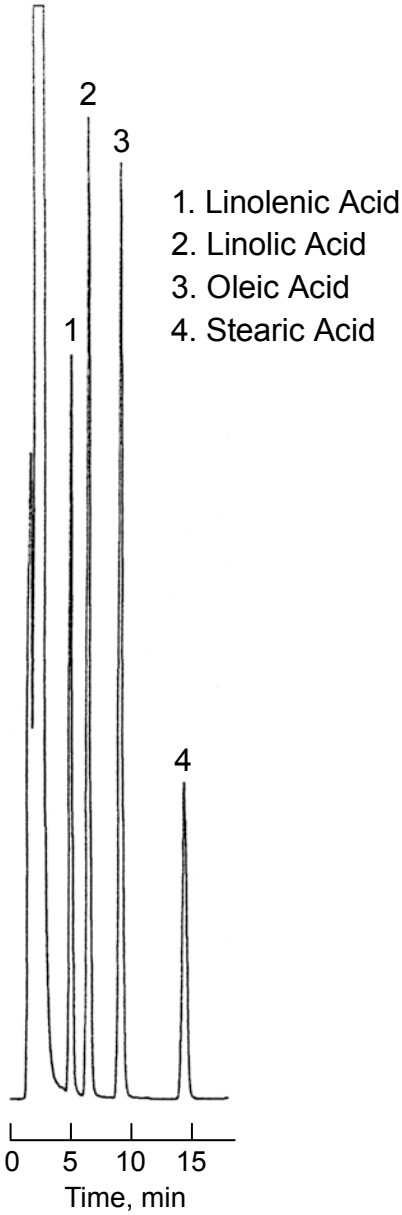
A5553	**3-Bromomethyl-7-methoxy-1,4-benzoxazin-2-one**	100 mg	1 g

References

1) H. Naganuma, A. Nakanishi, J. Kondo, K. Watanabe, Y. Kawahara, *Sankyo Kenkyusho Nempo* **1988**, *40*, 51.
2) A. Nakanishi, H. Naganuma, J. Kondo, K. Watanabe, Y. Kawahara, Program and Abstracts 109th Congress of the Pharmaceutical Society of Japan, 6TA, 2-1.
3) A. Nakanishi, H. Naganuma, J. Kondo, K. Watanabe, K. Hirano, T. Kawasaki, Y. Kawahara, *J. Chromatogr.* **1992**, *591*, 159.

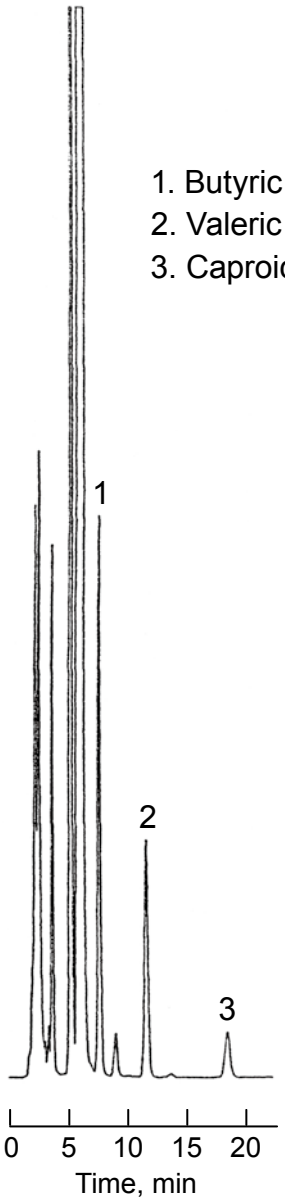
AZ-554

Chromatogram of fatty acids as 7-methoxy-1,4-benzoxazin-2-one-3-methyl ester



1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

Column	: Kaseisorb LC ODS-120-5 4.6 mmI.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 95 / 5
Detector	: Fluorescence λex 355 nm λem 430 nm
Temperature	: 30 °C
Flow Rate	: 1 mL / min

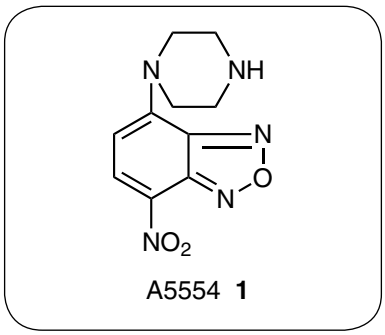


1. Butyric Acid(C_4)
2. Valeric Acid(C_5)
3. Caproic Acid(C_6)

Column	: Kaseisorb LC ODS-120-5 4.6 mmI.D.×150 mm
Mobile Phase	: CH_3CN / H_2O = 50 / 50
Detector	: Fluorescence λex 355 nm λem 430 nm
Temperature	: 25 °C
Flow Rate	: 1 mL / min

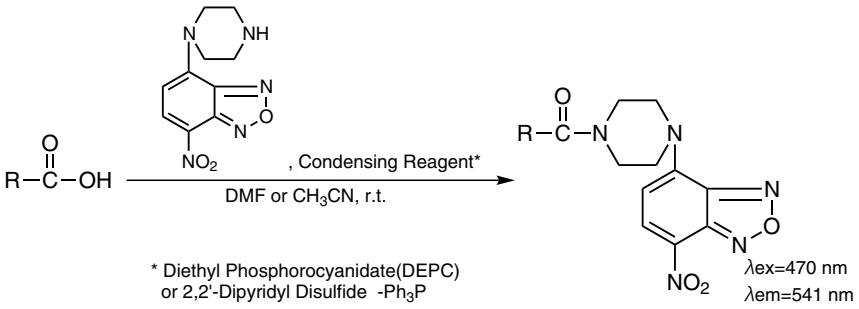
HPLC Labeling Reagent

for Carboxylic Acids



A5554 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a piperazino group, easily reacts with a carboxyl group at room temperature to form the corresponding amide in the presence of a condensation reagent. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 541 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done by using laser induced fluorescence detector.



Application example:

[Fatty acids] [1)]

0.2 mL of 140 mM DEPC or 70 mM 2,2'-dipyridyl disulfide-Ph_3P / DMF solution containing a fatty acid (10 μM) is added to 0.2 mL of the labeling reagent **1** / DMF or acetonitrile solution (10 mM). React at room temperature for 6 h, then use it as an HPLC sample.

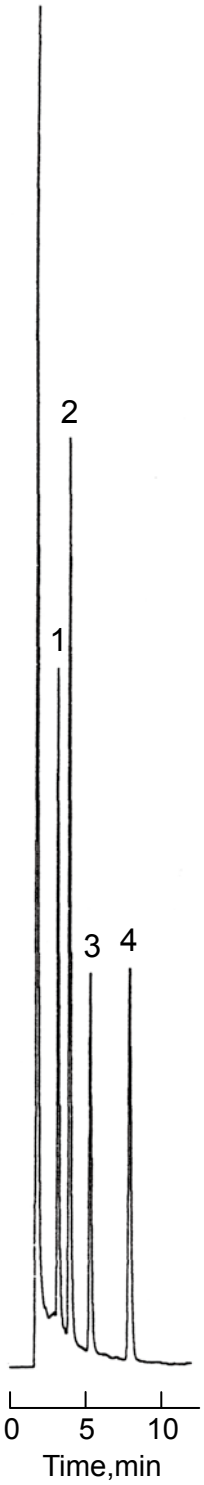
A5554	**NBD-PZ (=4-Nitro-7-piperazino-2,1,3-benzoxadiazole)**	100 mg

Reference

1) T. Toyo'oka, M. Ishibashi, Y. Takeda, K. Nakashima, S. Akiyama, S. Uzu, K. Imai, *J. Chromatogr.* **1991**, *588*, 61.

AZ-555

Chromatogram of fatty acids as NBD-PZ derivatives

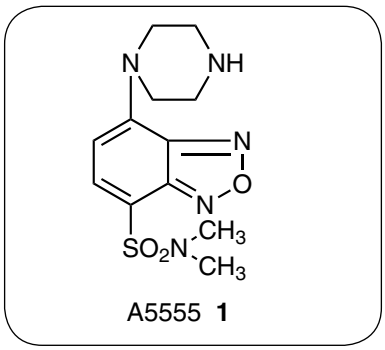


Column : Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN
Detector : Fluorescence λex 470 nm
λem 541 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

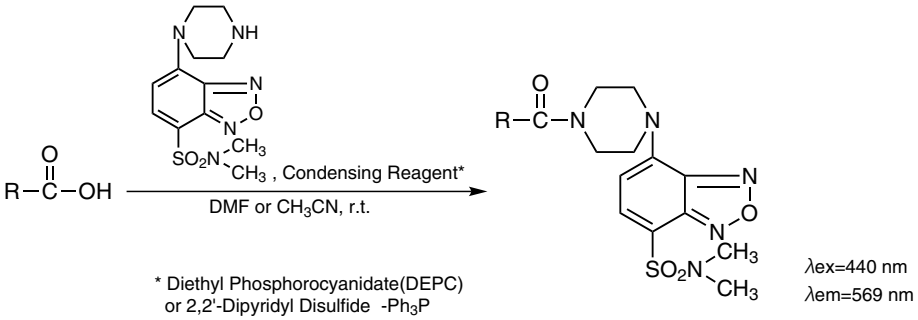
HPLC Labeling Reagent

for Carboxylic Acids



A5555 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a piperazino group, easily reacts with a carboxyl group at room temperature to form the corresponding amide in the presence of a condensation reagent. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 440 nm and 569 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection[1].



Application example:

[Fatty acids] [2]

0.2 mL of 140 mM DEPC or 70 mM 2,2'-dipyridyl disulfide-Ph_3P / DMF solution containing a fatty acid (10 μM) is added to 0.2 mL of the labeling reagent 1 / DMF or acetonitrile solution (10 mM). Incubate at room temperature for 6 h, then use it as an HPLC sample.

For example, the detection limit (S/N = 3) for saturated fatty acids (from C_{13} to C_{24}) is from 3.2 to 4.7 fmol.

A5555	**DBD-PZ** **[=4-(*N,N*-Dimethylaminosulfonyl)-7-piperazino-2,1,3-benzoxadiazole]**	100 mg

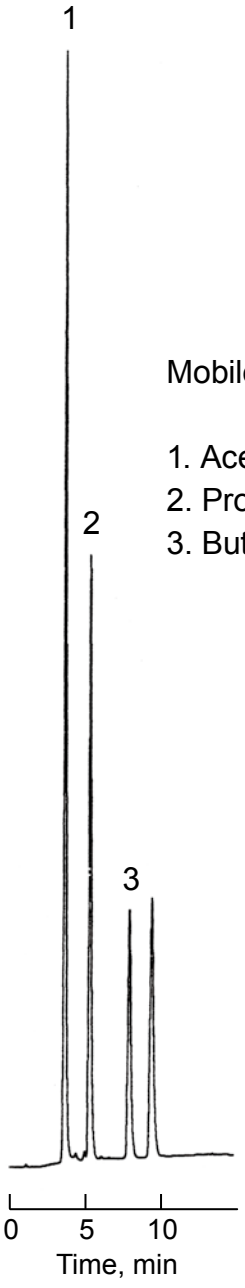
References

1) S. Uzu, K. Imai, K. Nakashima, S. Akiyama, Biomed. *Chromatogr.* **1991**, *5*, 184.
2) T. Toyo'oka, M. Ishibashi, Y. Takeda, K. Nakashima, S. Akiyama, S. Uzu, K. Imai, *J. Chromatogr.* **1991**, *588*, 61.

AZ-556

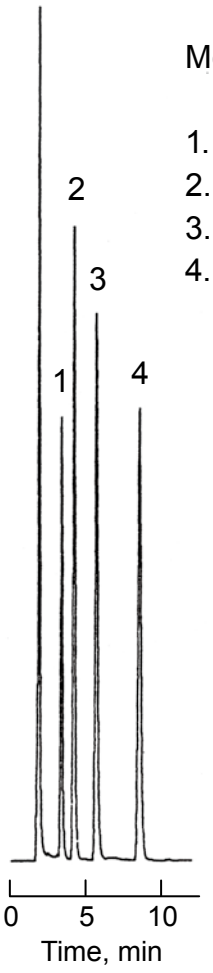
Chromatogram of fatty acids as DBD-PZ derivatives

Column : Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm
Detector : Fluorescence λex 440 nm λem 569 nm
Temperature : 25 °C
Flow Rate : 1 mL / min



Mobile Phase: CH_3CN / H_2O = 40 / 60

1. Acetic Acid
2. Propionic Acid
3. Butyric Acid

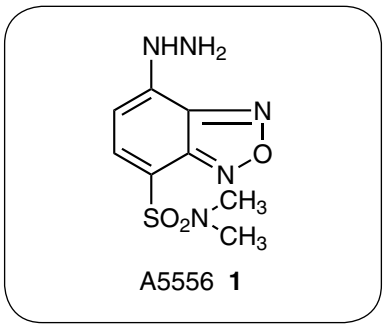


Mobile Phase: CH_3CN

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

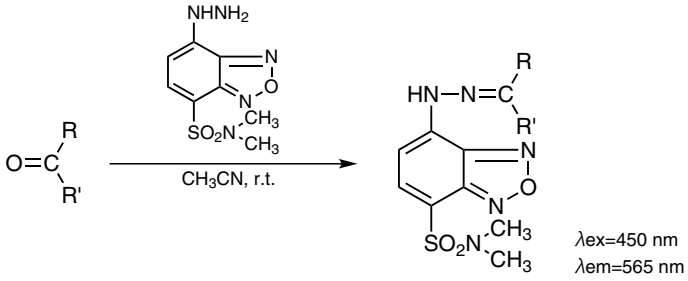
HPLC Labeling Reagent

for Carbonyl Compounds



A5556 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a hydrazino group, easily reacts with a carbonyl group to form the corresponding hydrazone. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 565 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done because of its strong fluorescence.



Application example:

[Aldehydes or ketones] [1)]

250 μM of the labeling reagent **1** and 1.7 μM propionaldehyde are added to acetonitrile containing 0.025% TFA, and reacted at room temperature for 30 min, then use it as the HPLC sample.

For example, the detection limit for propionaldehyde is 120 fmol.

A5556	**DBD-H** **[=4-(N,N-Dimethylaminosulfonyl)-7-hydrazino-2,1,3-benzoxadiazole)]**	100 mg

Reference

1) S. Uzu, S. Kanda, K. Imai, K. Nakashima, S. Akiyama, *Analyst* **1990**, *115*, 1477.

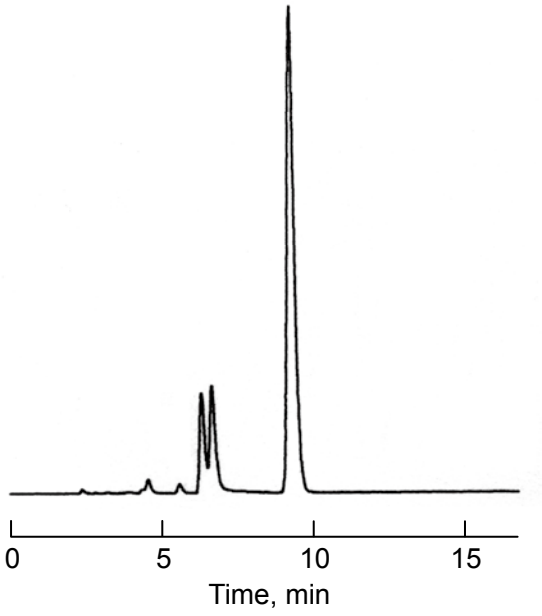
AZ-557

Chromatogram of aldehyde and ketone as DBD-H derivatives

Column	: Kaseisorb LC ODS Super	
	4.6 mmI.D.×150 mm	
Detector	: Fluorescence	λex 450 nm
		λem 565 nm
Temperature	: 25 ºC	
Flow Rate	: 1 mL / min	

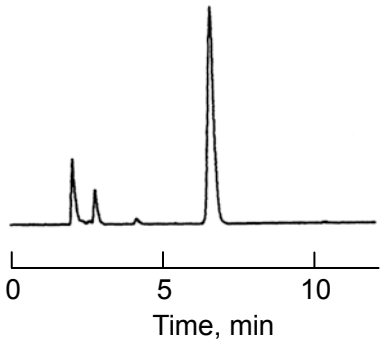
Mobile Phase :
CH_3CN / 0.05% TFA in H_2O
= 45 / 55

Propionaldehyde



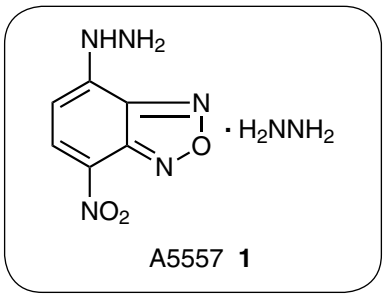
Mobile Phase :
CH_3CN / 0.05% TFA in H_2O
= 70 / 30

Heptan-2-one



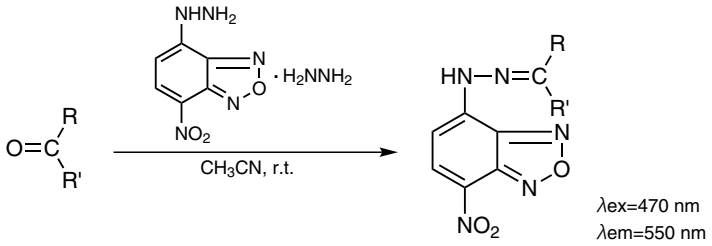
HPLC Labeling Reagent

for Carbonyl Compounds



A5557 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a hydrazino group, easily reacts with a carbonyl group to form the corresponding hydrazone. The labeling reagent itself is non-fluorescent, but the hydrazones after the reaction with carbonyl compounds have strong fluorescence. The resultant hydrazone is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 550 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants, and a highly sensitive detection can be done because of its high reactivity.



Application example:

[Aldehydes or ketones] [1)]

250 μM of the labeling reagent 1 and 1.7 μM propionaldehyde are added to acetonitrile containing 0.025% TFA, and reacted at room temperature for 1 h, use it as the HPLC sample.

For example, the detection limit for propionaldehyde is 35 fmol.

A5557	**NBD-H (4-Hydrazino-7-nitro-2,1,3-benzoxadiazole Hydrazine)**	100 mg

Reference

1) S. Uzu, S. Kanda, K. Imai, K. Nakashima, S. Akiyama, *Analyst* **1990**, *115*, 1477.

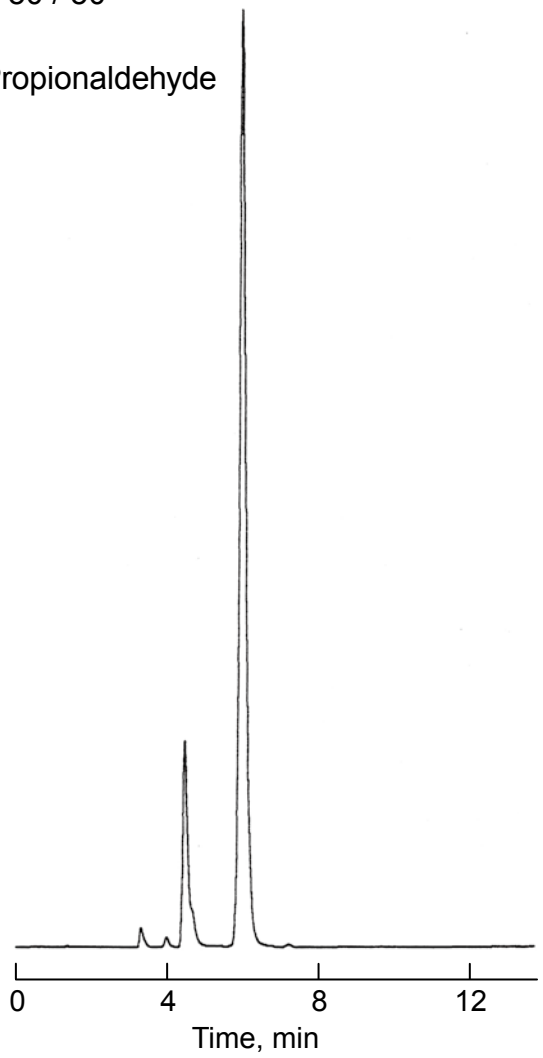
AZ-558

Chromatogram of aldehydes and ketones as NBD-H derivatives

Column : Kaseisorb LC ODS Super 4.6mmI.D.×150mm
Detector : Fluorescence λex 470 nm λem 550 nm
Temperature : 30 ºC
Flow Rate : 1mL / min

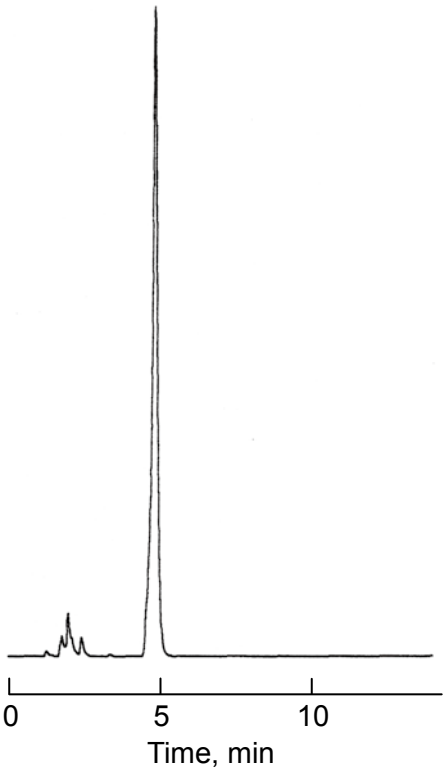
Mobile Phase :
CH_3CN / 0.05% TFA in H_2O
= 50 / 50

Propionaldehyde

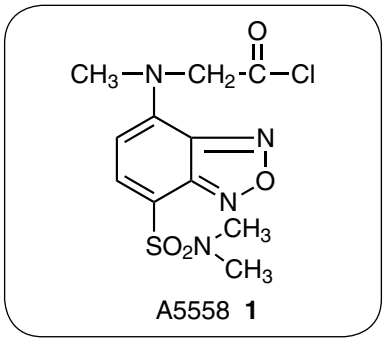


Mobile Phase :
CH_3CN / 0.05% TFA in H_2O
= 75 / 25

Heptan-2-one



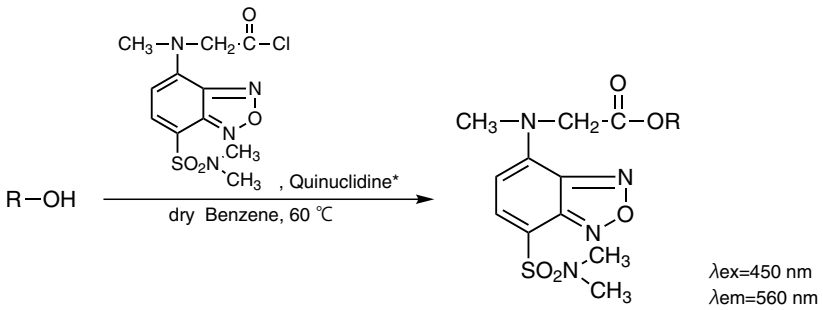
HPLC Labeling Reagent



The compound **1** is an HPLC fluorescence labeling reagent, which reacts with many kinds of nucleophilic groups under mild conditions. The reaction examples are shown in the table below.

These resulting compounds are stable, and can reach the detector without any decomposition under reversed phase HPLC, thus excellent chromatograms can be obtained by fluorescence detection.

Groups	Examples	Reaction Conditions	Wavelengths (nm)		Detection Limits(fmol)
			ex	em	
Alcohols	Androsterone	60 ºC, 30 min	443	546	38
α-Oxyacids	Mandelic acid	60 ºC, 15 min	442	551	125
Phenols	Estrone	60 ºC, 15 min	440	543	40
Amines	Benzylamine	r.t. or 60 ºC, 15 min	445	555	89
Aromatic amines	Phenetidine	60 ºC, 15 min	443	553	56
Thiols	2-Mercapto-*N*-(2-naphthyl)-acetamide	r.t.	437	544	103



Application example:

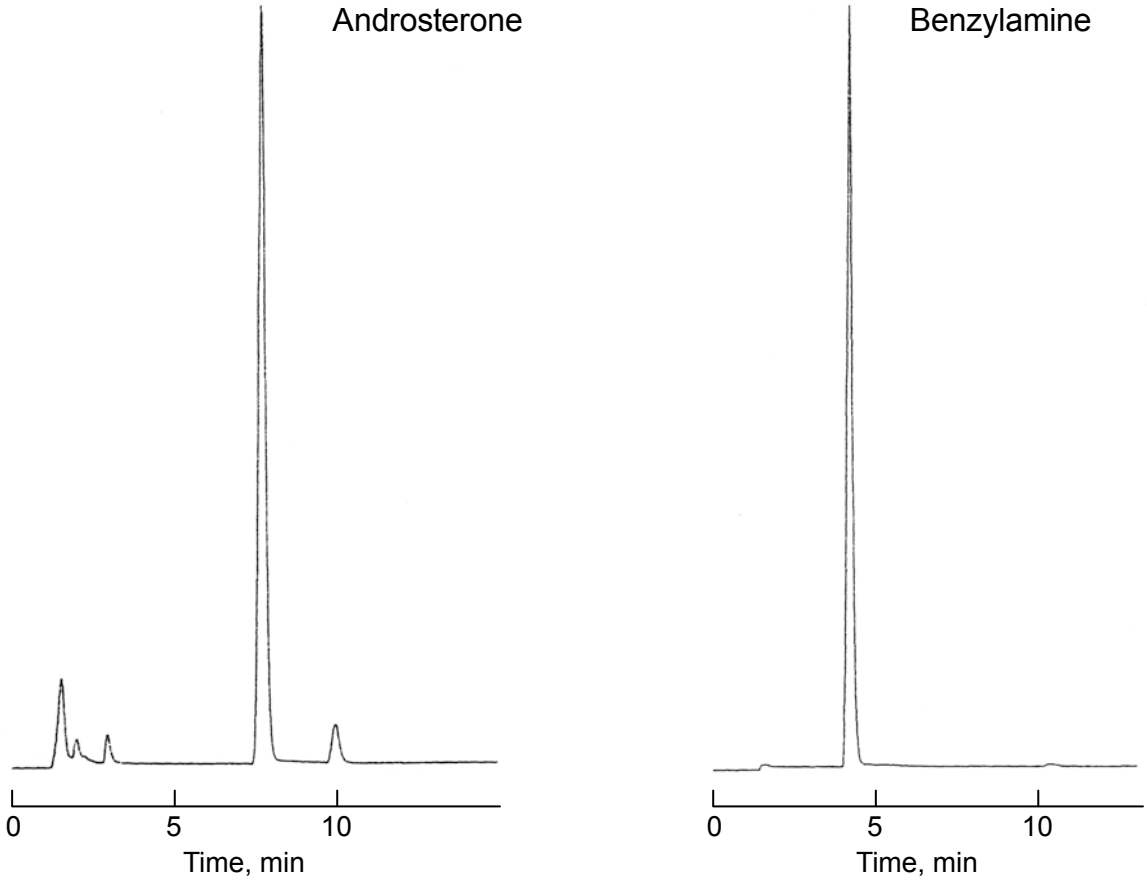
10 μL of 25 mM labeling reagent **1** in dry benzene is mixed with 10 μL of 0.5 mM androsterone in dry benzene (containing 0.5 mM quinuclidine*), and incubated at 60 ºC for 30 min. The reaction solution is quenched with 980 μL of 50% acetonitrile solution containing 1% acetic acid, use it as the HPLC sample solution.

*For primary alcohols, quinuclidine is not necessarily needed.

AZ-559

Chromatogram of alcohol and amine as DBD-COCl derivatives

Column : Kaseisorb LC ODS Super
4.6 mm I.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 50 / 50
Detector : Fluorescence λex 450 nm
λem 560 nm
Temperature : 40 °C
Flow Rate : 1 mL / min



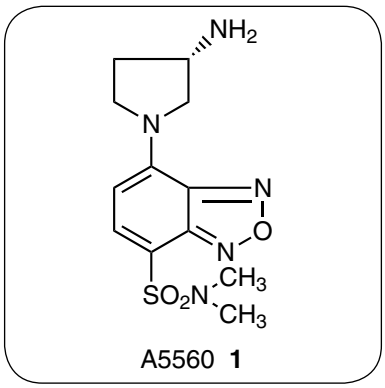
A5558 **DBD-COCl** 100 mg
[=4-(*N*,*N*-Dimethylaminosulfonyl)-7-(*N*-chloroformylmethyl-*N*-methylamino)-2,1,3-benzoxadiazole]

References

1) K. Imai, T. Fukushima, H. Yokosu, *Biomed. Chromatogr.* **1994**, *8*, 107.
2) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 95 238075, 1995.

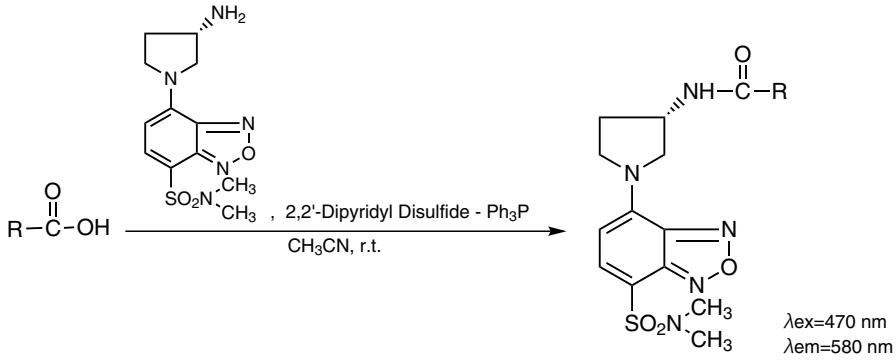
HPLC Labeling Reagent

for Chiral Carboxylic Acids



A5560 **1**

The compound **1** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 580 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection.



Application example:[1)]

Add 0.1 mL of 10 mM labeling reagent **1** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 10 fmol.

A5560	**(*S*)-(+)-DBD-APy** **[=(*S*)-(+)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

References

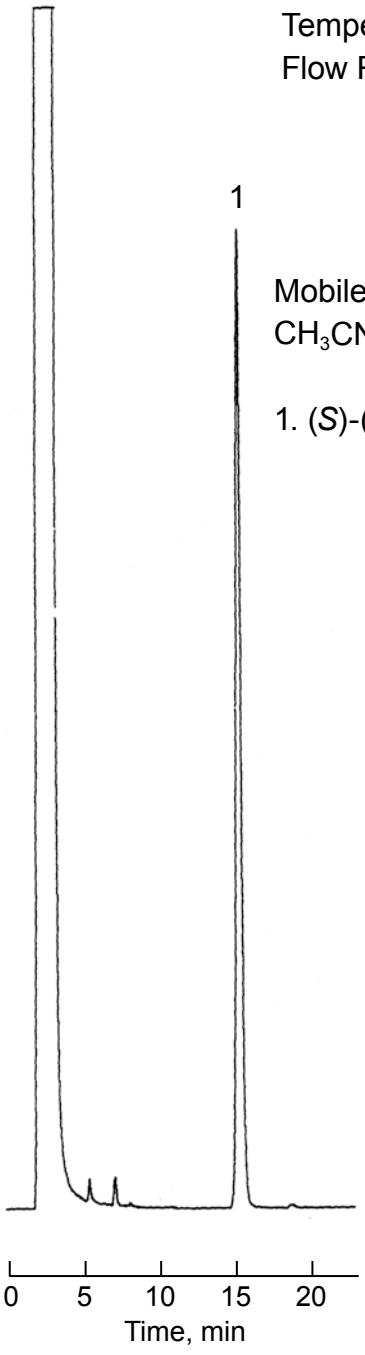
1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.

2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr.* **1992**, *625*, 357.

AZ-561

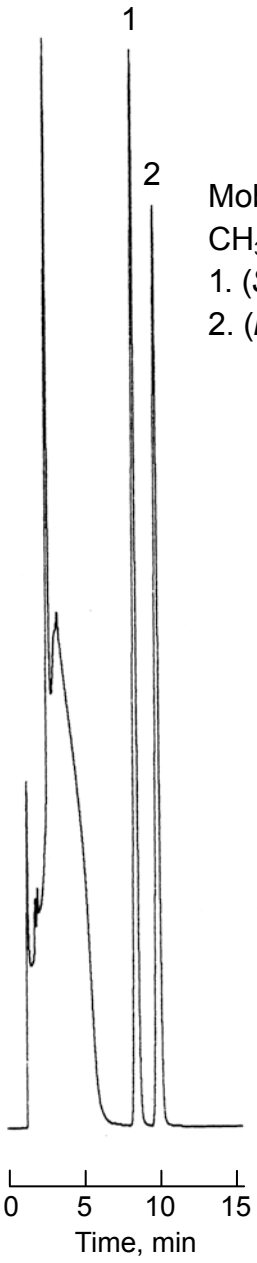
Chromatogram of carboxylic acid enantiomers as (*S*)-(+)-DBD-APy derivatives

Column : Kaseisorb LC ODS Super
4.6 mmI.D.×150 mm
Detector : Fluorescence λex 470 nm
λem 580 nm
Temperature : 40 ºC
Flow Rate : 1 mL / min



Mobile Phase :
CH_3CN / H_2O = 50 / 50

1. (*S*)-(+)-Naproxen

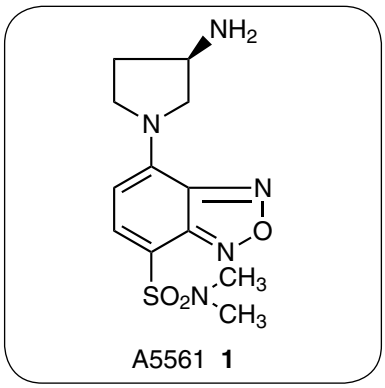


Mobile Phase :
CH_3CN / H_2O = 60 / 40

1. (*S*)-(+)-Ibuprofen
2. (*R*)-(-)-Ibuprofen

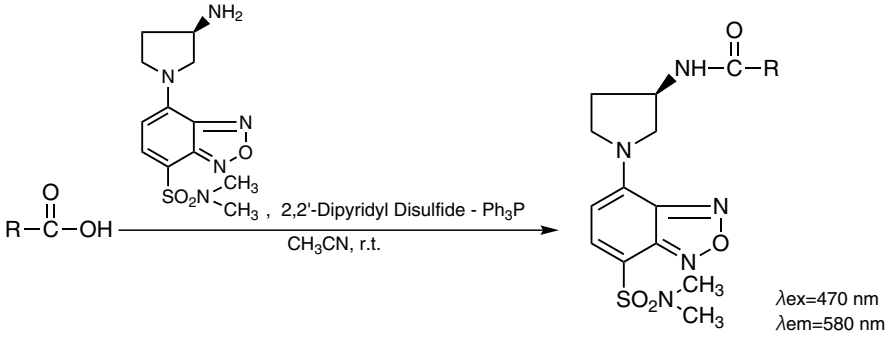
HPLC Labeling Reagent

for Chiral Carboxylic Acids



A5561 **1**

The compound **1** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 580 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection.



Application example:[1)]

Add 0.1 mL of 10 mM labeling reagent **1** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 10 fmol.

A5561	**(*R*)-(-)-DBD-APy** **[=(*R*)-(-)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

References

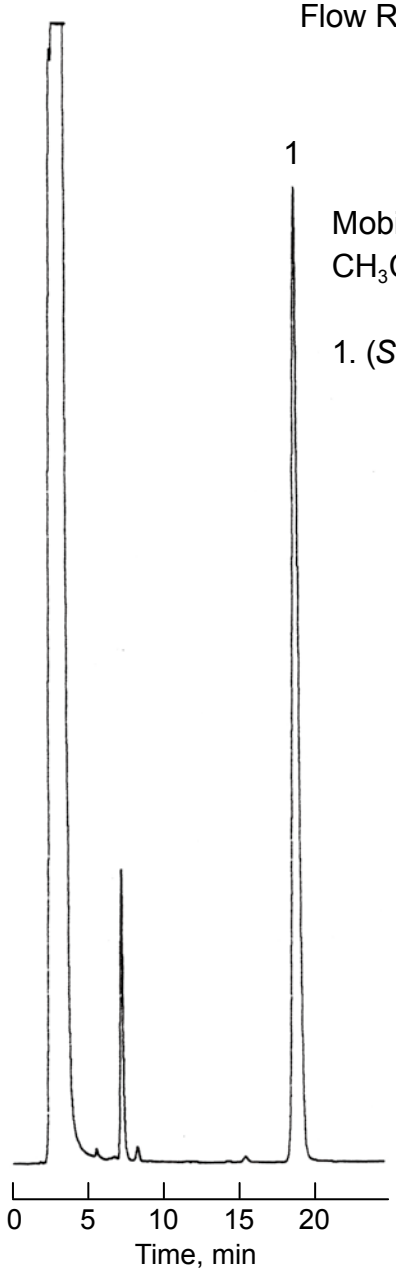
1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.

2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr.* **1992**, *625*, 357.

AZ-562

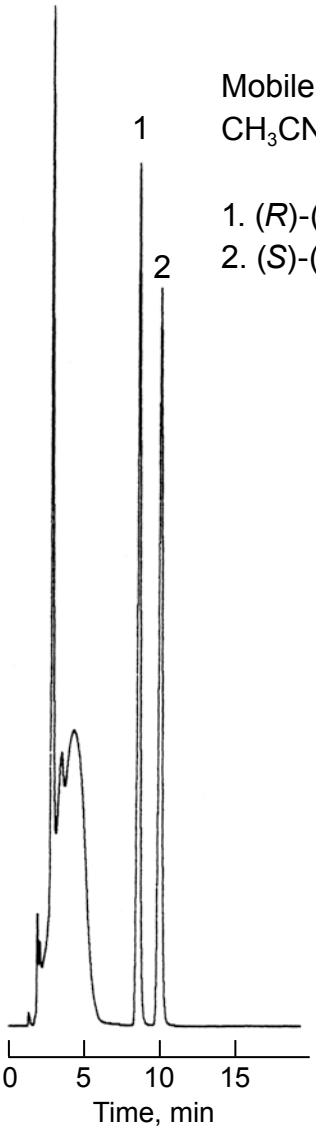
Chromatogram of carboxylic acid enantiomers as (*R*)-(-)-DBD-APy derivatives

Column : Kaseisorb LC ODS Super
4.6 mmI.D.×150 mm
Detector : Fluorescence λex 470 nm
λem 580 nm
Temperature : 40 ºC
Flow Rate : 1 mL / min



Mobile Phase :
CH_3CN / H_2O = 50 / 50

1. (*S*)-(+)-Naproxen

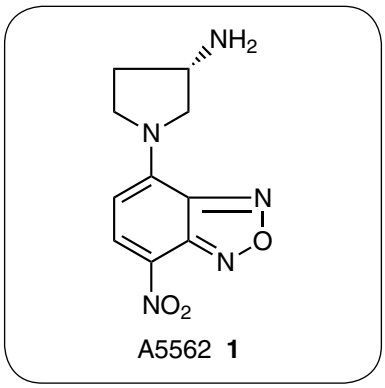


Mobile Phase :
CH_3CN / H_2O = 60 / 40

1. (*R*)-(-)-Ibuprofen
2. (*S*)-(+)-Ibuprofen

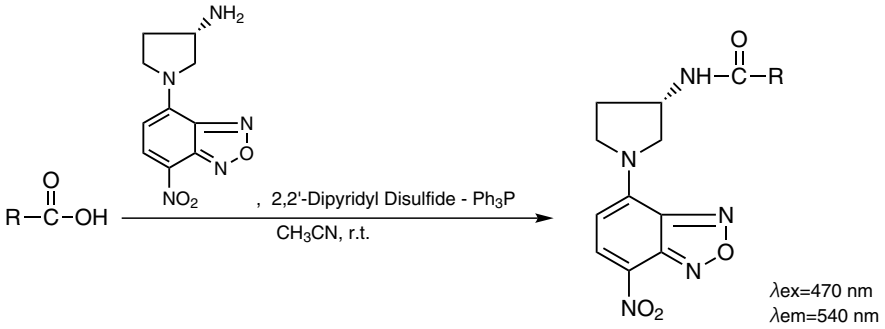
HPLC Labeling Reagent

for Chiral Carboxylic Acids



A5562 **1**

The compound **1** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done by using laser induced fluorescence detector.



Application example:[2)]

Add 0.1 mL of 10 mM labeling reagent **1** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 15 fmol.

A5562	**(*S*)-(+)-NBD-APy** **[=(*S*)-(+)-4-Nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

References

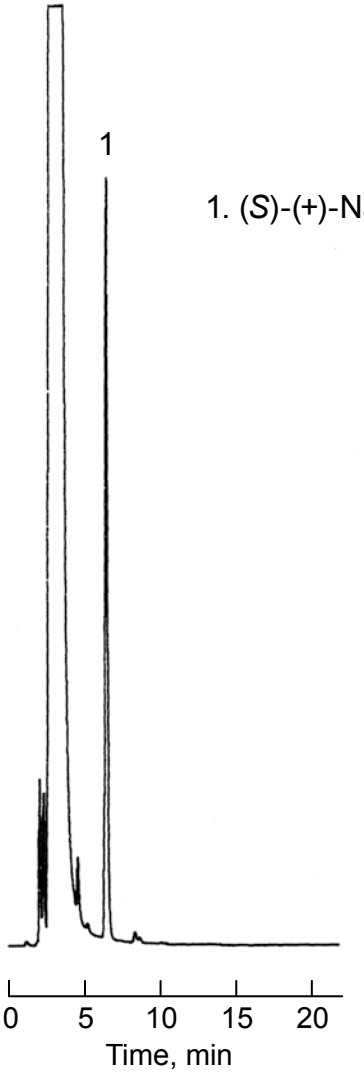
1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.

2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr.* **1992**, *625*, 357.

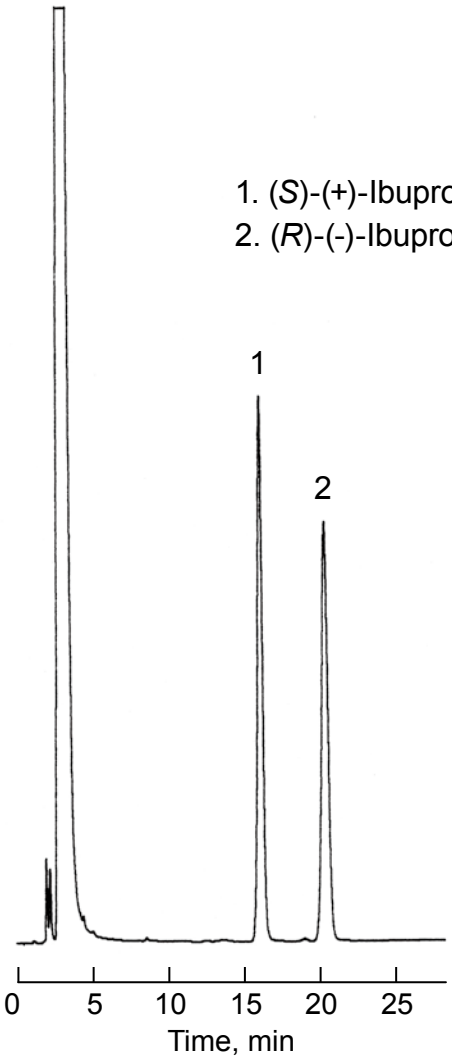
AZ-563

Chromatogram of carboxylic acid enantiomers as (*S*)-(+)-NBD-APy derivatives

Column : Kaseisorb LC ODS Super
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 50 / 50
Detector : Fluorescence λex 470 nm
λem 540 nm
Temperature : 40 ºC
Flow Rate : 1 mL / min



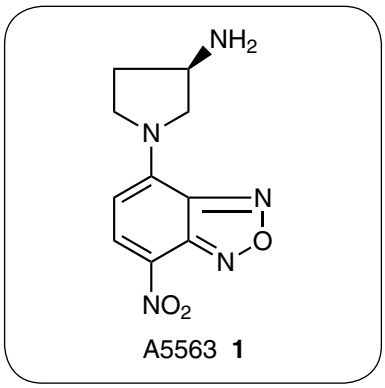
1. (*S*)-(+)-Naproxen



1. (*S*)-(+)-Ibuprofen
2. (*R*)-(-)-Ibuprofen

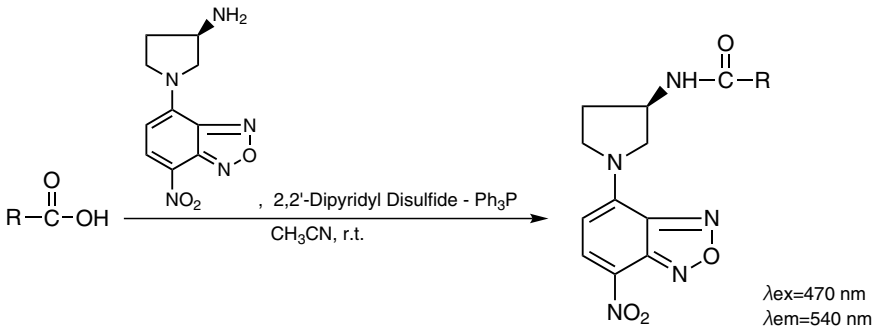
HPLC Labeling Reagent

for Chiral Carboxylic Acids



A5563 **1**

The compound **1** is an optically active HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, and is used for optical purity determination of carboxylic acids. Labeling of racemic carboxylic acids can be done by using a mild condition such as the Mukaiyama-Corey method, and produces diastereomers without inducing racemization. These diastereomers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. A highly sensitive detection can be done by using laser induced fluorescence detector.



Application example:[2)]

Add 0.1 mL of 10 mM labeling reagent **1** / acetonitrile solution, 0.25 mL of 2 μM carboxylic acid / acetonitrile solution, and 0.15 mL of 10 mM 2,2'-dipyridyl disulfide-triphenylphosphine / acetonitrile solution to a vessel, and react the mixture at room temperature for 4 h. Use the resultant as an HPLC sample solution.

For example, the detection limit (S/N=2) for naproxen is 15 fmol.

A5563	**(*R*)-(-)-NBD-APy** **[=(*R*)-(-)-4-Nitro-7-(3-aminopyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

References

1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1992**, *117*, 727.

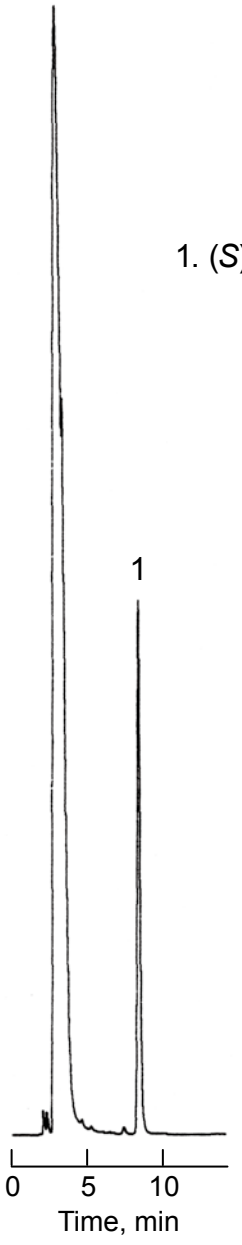
2) T. Toyo'oka, M. Ishibashi, T. Terao, *J. Chromatogr.* **1992**, *625*, 357.

AZ-564

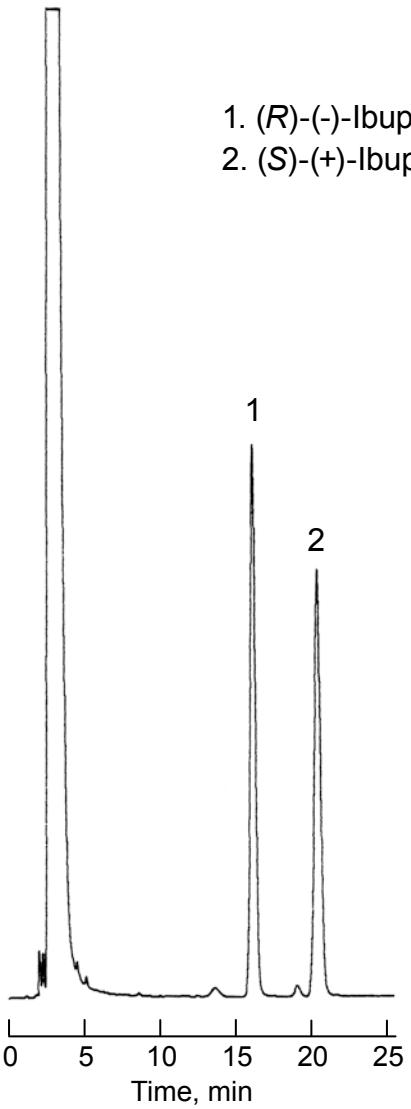
Chromatogram of carboxylic acid enantiomers as (*R*)-(-)-NBD-APy derivatives

Column : Kaseisorb LC ODS Super
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 50 / 50
Detector : Fluorescence λex 470 nm
λem 540 nm
Temperature : 40 °C
Flow Rate : 1 mL / min

1. (*S*)-(+)-Naproxen

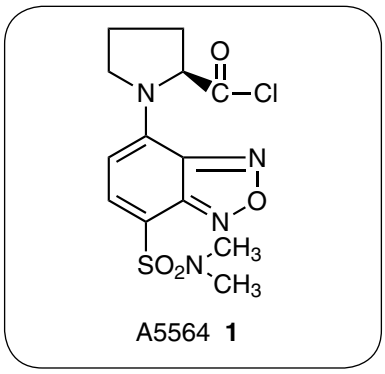


1. (*R*)-(-)-Ibuprofen
2. (*S*)-(+)-Ibuprofen



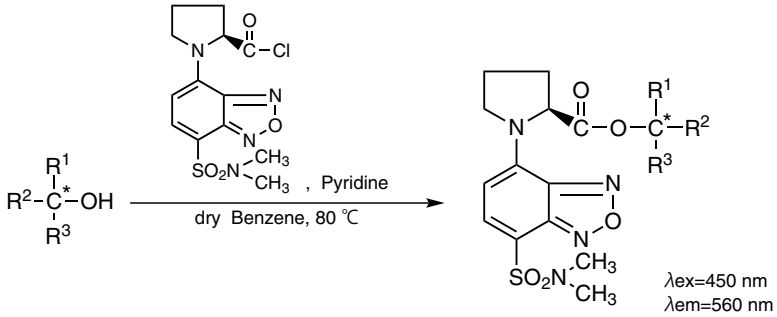
HPLC Labeling Reagent

for Chiral Alcohols and Amines



A5564 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 560 nm, respectively. And the diastereomers derived from racemic alcohol and **1** can be separated by HPLC (The separation factor α: 2-hexanol = 1.2). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*R*)-(+)-DBD-Pro-COCl] of **1**. The detection limit for the alcohols is sub-picomol. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection.



Application example:

[Secondary alcohols] [1)]

Add 1 mL of 10 mM labeling reagent **1** / dry benzene solution and 1 mL of 2 mM alcholol / dry benzene (containing 1% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 ºC for 3 h. After cooling to room temperature, excess of **1** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.

A5564	**(*S*)-(-)-DBD-Pro-COCl** **[=(*S*)-(-)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

References

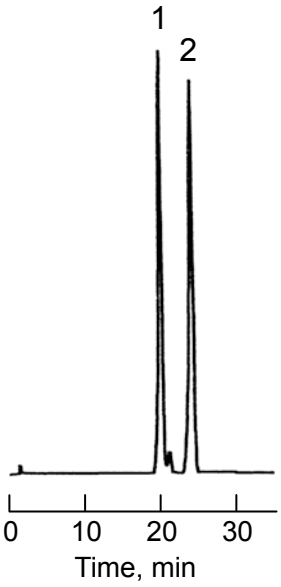
1) T. Toyo'oka, M. Ishibashi, T. Terao, *Analyst* **1993**, *118*, 759.

2) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 94 184141, **1994**.

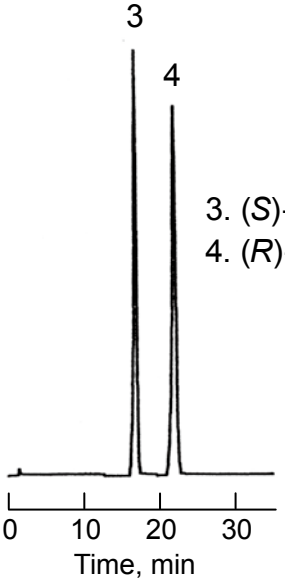
AZ-565

Chromatogram of alcohol enantiomers as (*S*)-(-)-DBD-Pro-COCl derivatives

Column : Kaseisorb LC 60-5
4.6 mmI.D.×150 mm
Mobile Phase : Hexane / AcOEt = 80 / 20
Detector : Fluorescence λex 450 nm
λem 560 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min

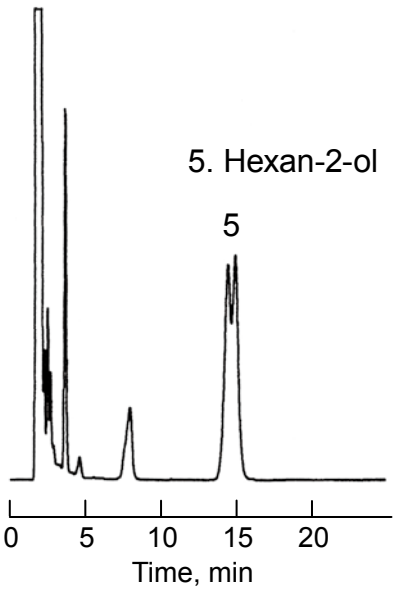


1. (*S*)-(+)-Hexan-2-ol
2. (*R*)-(-)-Hexan-2-ol



3. (*S*)-(+)- Nonan-2-ol
4. (*R*)-(-)-Nonan-2-ol

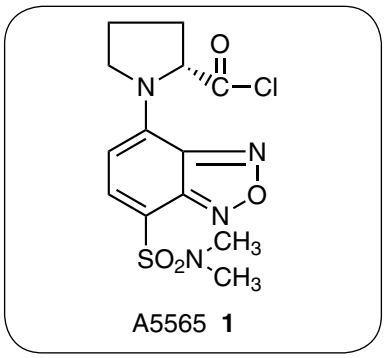
Column : Kaseisorb LC ODS Super
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 60 / 40
Detector : Fluorescence λex 450 nm
λem 560 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min



5. Hexan-2-ol

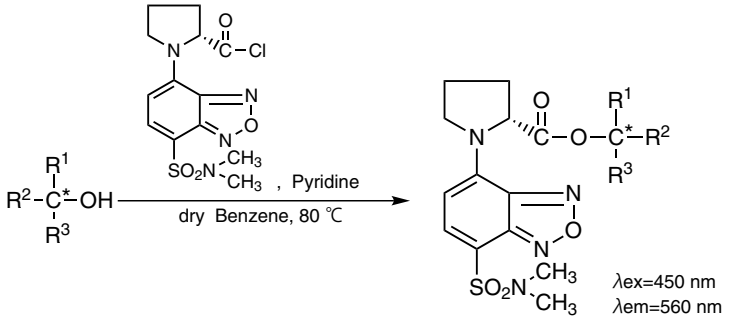
HPLC Labeling Reagent

for Chiral Alcohols and Amines



A5565 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelength of 450 nm and 560 nm, respectively. And the diastereomers derived from racemic alcohol and **1** can be separated by HPLC (The separation factor α: 2-hexanol = 1.2). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*S*)-(-)-DBD-Pro-COCl] of **1**. The detection limit for the alcohols is sub-picomol. A highly sensitive analysis can be done by peroxyoxalate chemiluminescence detection.



Application example:

[Secondary alcohols] [1)]

Add 1 mL of 10 mM labeling reagent **1** / dry benzene solution, 1 mL of 2 mM alcholol / dry benzene (containing 1% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 °C for 3 h. After cooling to room temperature, excess of **1** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.

A5565	**(*R*)-(+)-DBD-Pro-COCl** **[=(*R*)-(+)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

References

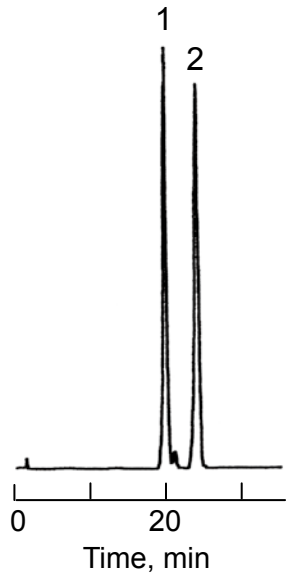
1) T. Toyo'oka, M. Ishibashi, T. Terao, K. Imai, *Analyst* **1993**, *118*, 759.

2) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 94 184141, **1994**.

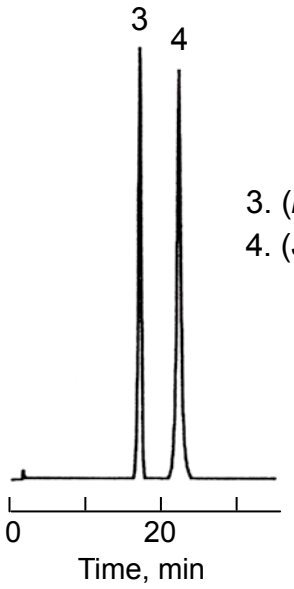
AZ-566

Chromatogram of alcohol enantiomers as (*R*)-(+)-DBD-Pro-COCl derivatives

Column : Kaseisorb LC 60-5
4.6 mmI.D.×150 mm
Mobile Phase : Hexane / AcOEt = 80 / 20
Detector : Fluorescence λex 450 nm
λem 560 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min

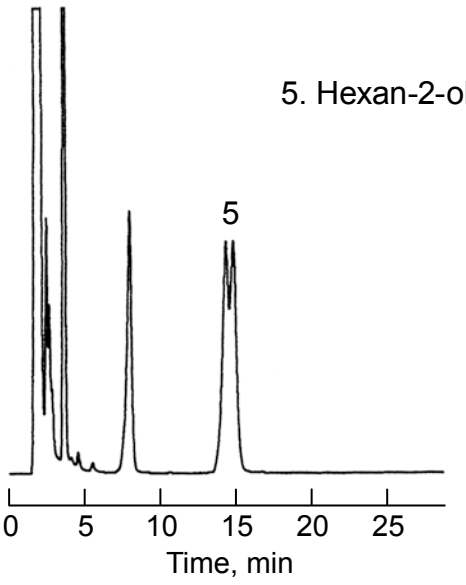


1. (*R*)-(-)-Hexan-2-ol
2. (*S*)-(+)-Hexan-2-ol



3. (*R*)-(-)-Nonan-2-ol
4. (*S*)-(+)- Nonan-2-ol

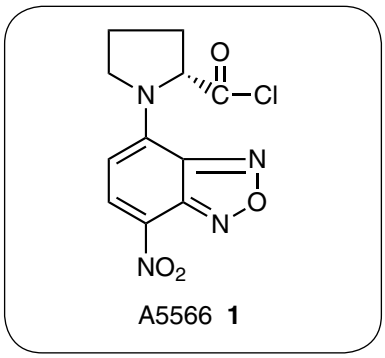
Column : Kaseisorb LC ODS Super
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 60 / 40
Detector : Fluorescence λex 450 nm
λem 560 nm
Temperature : 25 ºC
Flow Rate : 1 mL / min



5. Hexan-2-ol

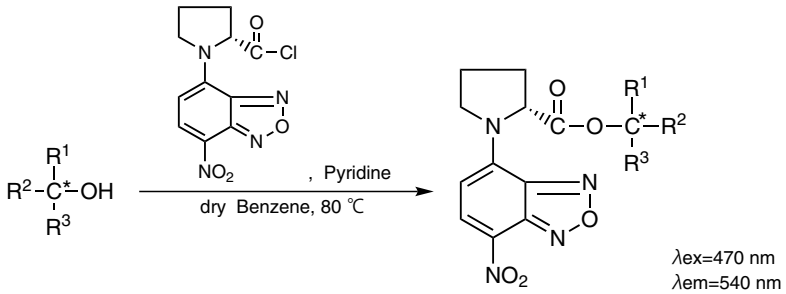
HPLC Labeling Reagent

for Chiral Alcohols and Amines



A5566 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. And the diastereomers derived from racemic alcohol or amine and **1** can be separated by HPLC (The separation factor α: 2-hexanol and 1-phenylethylamine = 1.2 and 1.37, respectively). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*S*)-(-)-NBD-Pro-COCl] of **1**. The detection limit for the alcohols is sub-picomol. A highly sensitive detection can be done by using laser induced fluorescence detector.



Application example:[1)]

Add 0.5 mL of 40 mM labeling reagent **1** / dry benzene solution and 0.5 mL of 1 mM alcholol (or amine) / dry benzene (containing 2% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 ºC for 1~2 h (50 ºC for 1h, in the case of amine). After cooling to room temperature, excess of **1** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.

A5566	**(*R*)-(+)-NBD-Pro-COCl** **[=(*R*)-(+)-4-Nitro-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

Reference

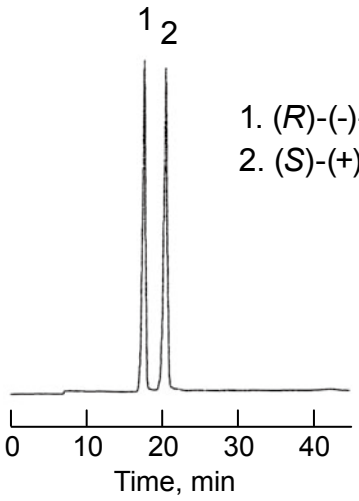
1) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 95 188224, **1995**.

AZ-567

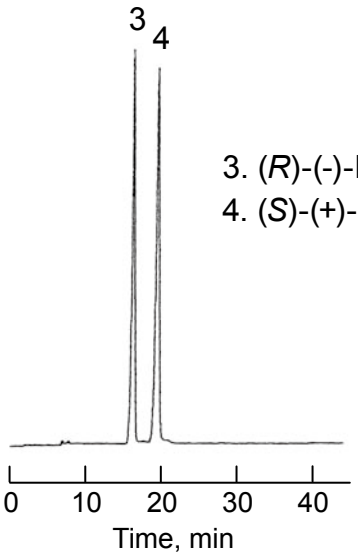
Chromatogram of alcohol and amine enantiomers as (*R*)-(+)-NBD-Pro-COCl derivatives

Column : Kaseisorb LC 60-5
4.6 mmI.D.×150 mm
Mobile Phase : Hexane / AcOEt = 80 / 20
Detector : Fluorescence λex 470 nm
λem 540 nm

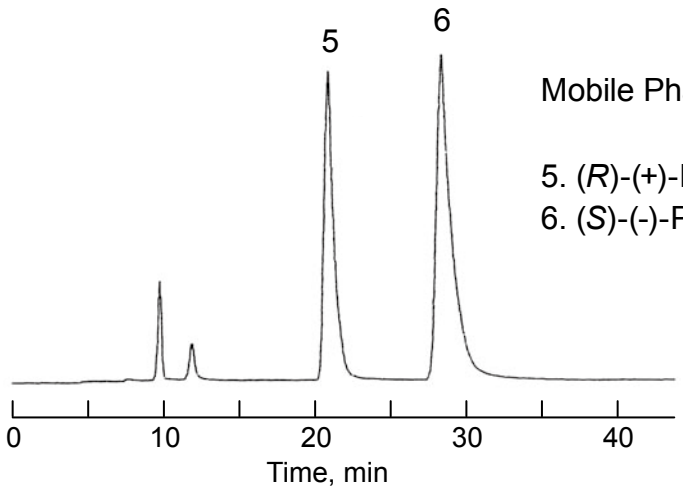
Temperature : 40 ºC
Flow Rate : 1 mL / min



1. (*R*)-(-)-Hexan-2-ol
2. (*S*)-(+)-Hexan-2-ol



3. (*R*)-(-)-Heptan-2-ol
4. (*S*)-(+)-Heptan-2-ol

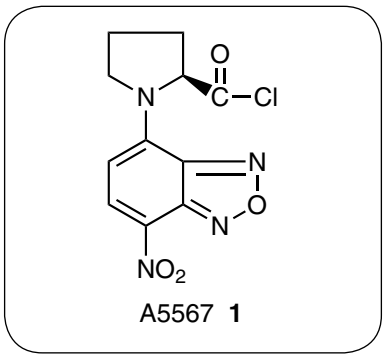


Mobile Phase : Hexane / AcOEt = 55 / 45

5. (*R*)-(+)-Phenylethylamine
6. (*S*)-(-)-Phenylethylamine

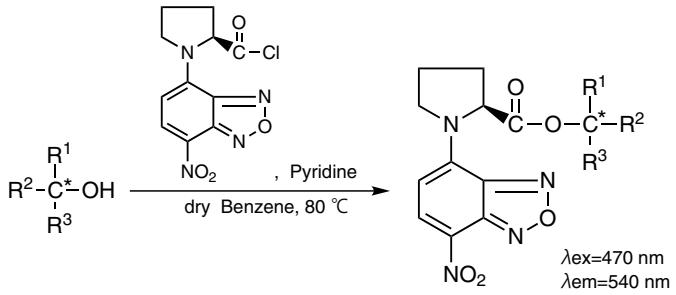
HPLC Labeling Reagent

for Chiral Alcohols and Amines



A5567 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination, which easily reacts with optical active alcohols or amines to form the corresponding esters or amides, respectively. The resultant esters or amides are stable and can reach the detector without any decomposition under reversed and normal phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 540 nm, respectively. And the diastereomers derived from racemic alcohol or amine and **1** can be separated by HPLC (The separation factor α: 2-hexanol and 1-phenylethylamine = 1.2 and 1.37, respectively). Since no racemization can occur with derivatization reaction, it is possible to change an elution order of the labeled diastereomeres by selecting the enantiomer [(*R*)-(+)-NBD-Pro-COCl] of **1**. The detection limit for the alcohols is sub-picomol. A highly sensitive detection can be done by laser induced fluorescence detector.



Application example:[1)]

Add 0.5 mL of 40 mM labeling reagent **1** / dry benzene solution, 0.5 mL of 1 mM alcholol (or amine) / dry benzene (containing 2% of pyridine) solution to a vessel. Close the cap of the reaction vessel and incubate the mixture at 80 ºC for 1~2 h (50 ºC for 1 h, in the case of amine). After cooling to room temperature, excess of **1** is removed by liquid-liquid extraction (e.g. washing with 5% $NaHCO_3$ solution) or solid phase extraction. Use the resultant as an HPLC sample solution.

A5567	**(*S*)-(-)-NBD-Pro-COCl** **[=(*S*)-(-)-4-Nitro-7-(2-chloroformylpyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

Reference

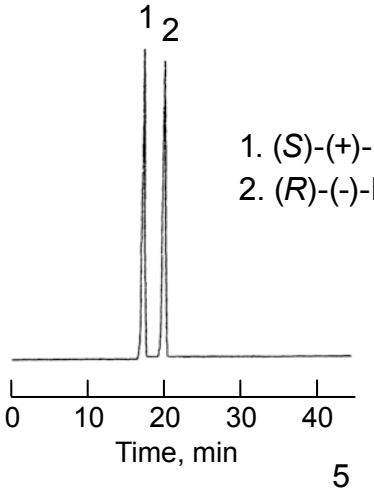
1) Tokyo Kasei Kogyo Co. Ltd., Jpn. Kokai Tokkyo Koho 95 188224, **1995**.

AZ-568

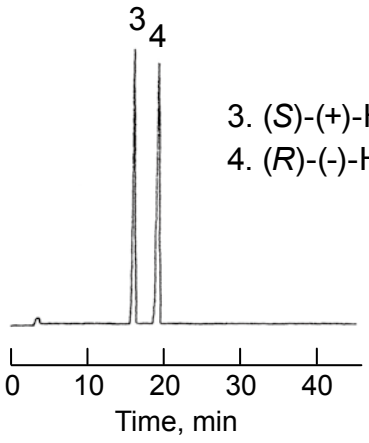
Chromatogram of alcohol and amine enantiomers as (*S*)-(-)-NBD-Pro-COCl derivatives

Column : Kaseisorb LC 60-5
4.6 mmI.D.×150 mm
Mobile Phase : Hexane / AcOEt = 80 / 20
Detector : Fluorescence λex 470 nm
λem 540 nm

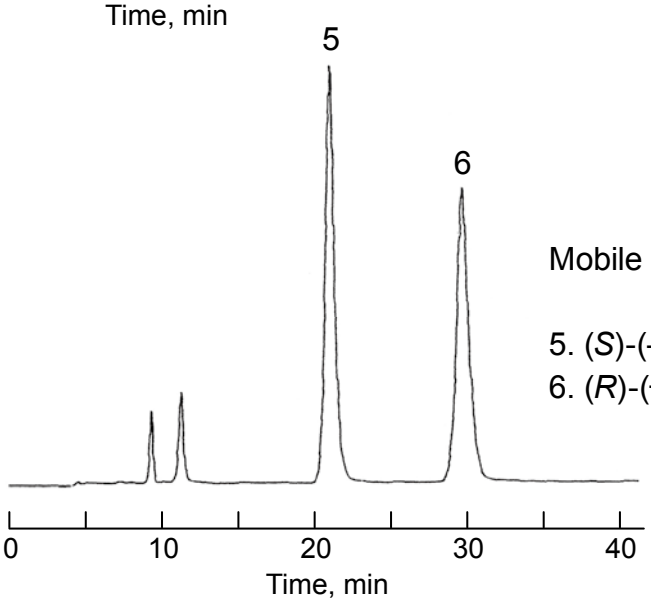
Temperature : 40 ºC
Flow Rate : 1 mL / min



1. (*S*)-(+)-Hexan-2-ol
2. (*R*)-(-)-Hexan-2-ol



3. (*S*)-(+)-Heptan-2-ol
4. (*R*)-(-)-Heptan-2-ol

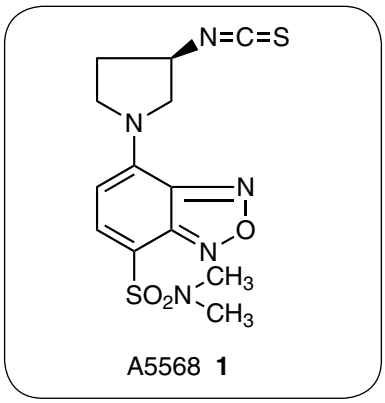


Mobile Phase : Hexane / AcOEt = 55 / 45

5. (*S*)-(-)-Phenylethylamine
6. (*R*)-(+)-Phenylethylamine

HPLC Labeling Reagent

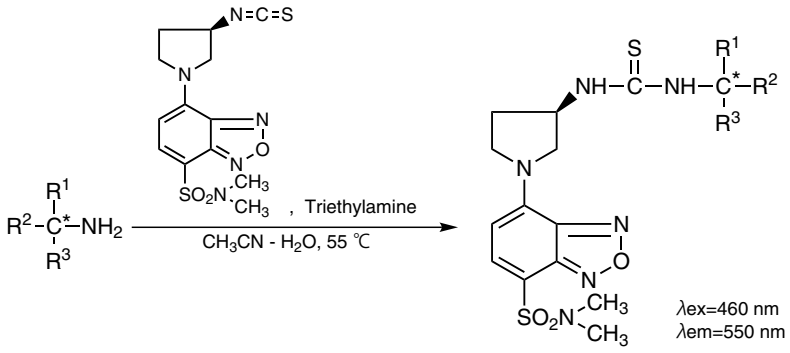
for Chiral Amines and Thiols



A5568 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino or mercapto groups, which are directly linked to the asymmetric carbon atom and produces diastereomers of thiourea or dithiocarbamate. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 460 nm and 550 nm, respectively. [The detection limit: thiopronine = 0.5 pmol (S/N = 2)]

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change an elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with a high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.



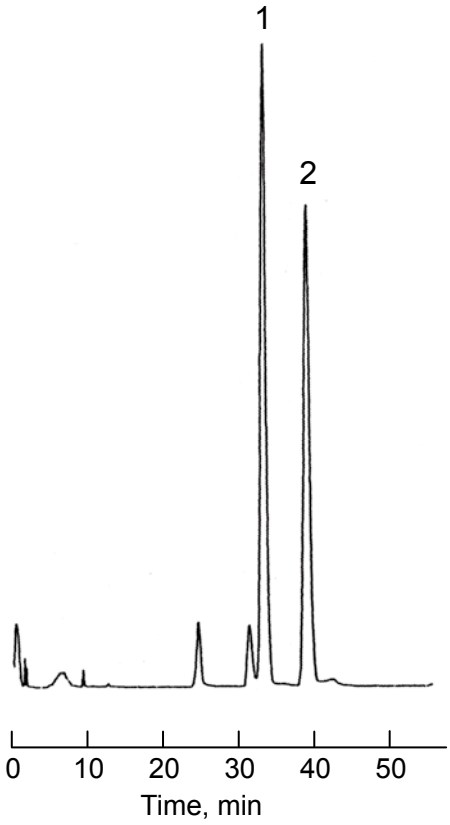
Application example:

Add 10 μL of 5 mM HPLC labeling reagent **1** / acetonitrile solution in 10 μL of 1 mM amine / acetonitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel, close the cap of the reaction vessel and incubate the mixture at 55 ºC for 10 min. Then, add 480 μL of a mixture solution of 1 M acetic acid and acetonitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 μL of this diluted solution as an HPLC sample solution.

A5568	**(*R*)-(-)-DBD-Py-NCS** **[=(*R*)-(-)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole]**	100 mg

AZ-569

Chromatogram of amines as (*R*)-(-)-DBD-Py-NCS derivatives



Column : Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 40 / 60 containing 0.05% TFA
Detector : Fluorescence λex 460 nm
λem 550 nm
Temperature : Ambient
Flow Rate : 1 mL / min

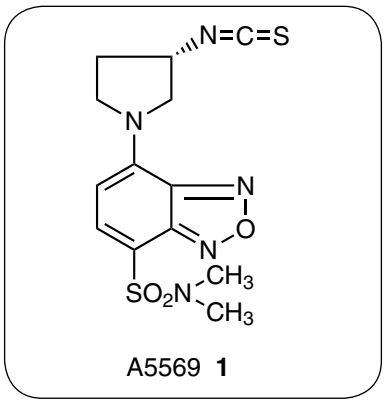
1. (*R*)-1-Phenylethylamine
2. (*S*)-1-Phenylethylamine

References

1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.
5) D. Jin, K. Takehana, T. Toyo'oka, *Anal. Sci.* **1997**, *13*, 113.

HPLC Labeling Reagent

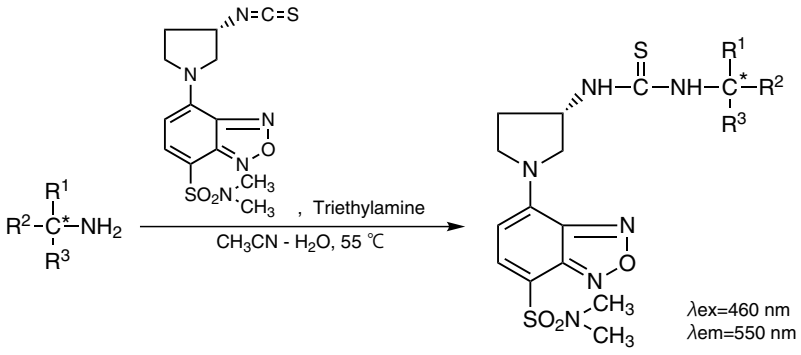
for Chiral Amines and Thiols



A5569 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino or mercapto groups, which are directly linked to the asymmetric carbon atom and produces diastereomers of thiourea or dithiocarbamate. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 460 nm and 550 nm, respectively. [The detection limit: thiopronine = 0.5 pmol (S/N = 2)]

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change an elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with a high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.



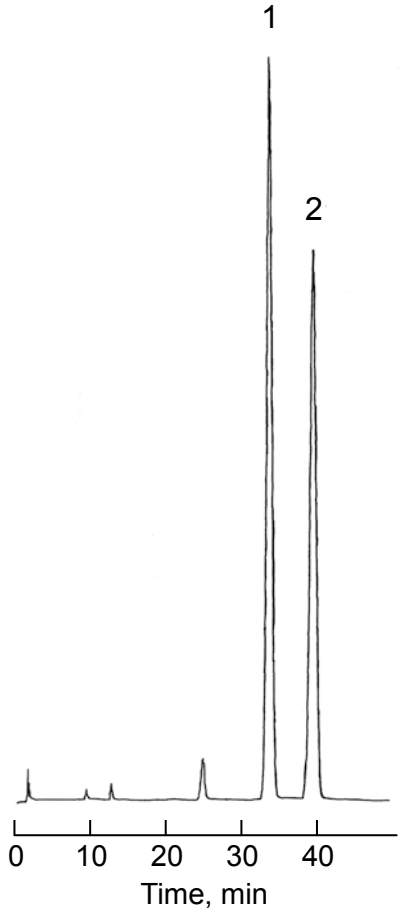
Application example:

Add 10 μL of 5 mM HPLC labeling reagent **1** / acetonitrile solution in 10 μL of 1 mM amine / acetnitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel, close the cap of the reaction vessel and incubate the mixture at 55 °C for 10 min. Then, add 480 μL of a mixture solution of 1 M acetic acid and acetnitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 μL of this diluted solution as an HPLC sample solution.

A5569 **(*S*)-(+)-DBD-Py-NCS** 100 mg
[=(*S*)-(+)-4-(*N*,*N*-Dimethylaminosulfonyl)-7-(3-isothiocyanatopyrrolidin-1-yl)-2,1,3-benzoxadiazole]

AZ-570

Chromatogram of amines as (*S*)-(+)-DBD-Py-NCS derivatives



Column : Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 40 / 60 containing 0.05% TFA
Detector : Fluorescence λex 460 nm
λem 550 nm
Temperature : Ambient
Flow Rate : 1.0 mL / min

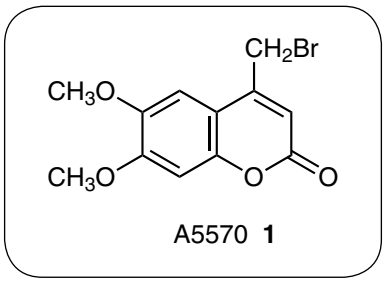
1. (*S*)-1-Phenylethylamine
2. (*R*)-1-Phenylethylamine

References

1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.
5) D. Jin, K. Takehana, T. Toyo'oka, *Anal. Sci.* **1997**, *13*, 113.

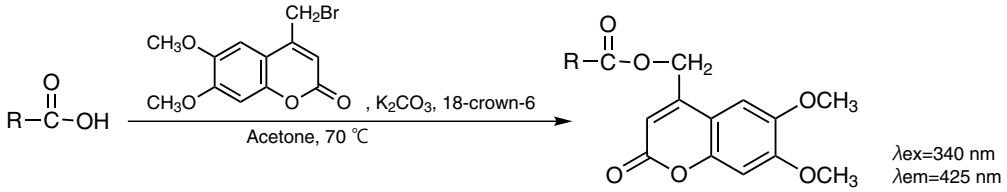
HPLC Labeling Reagent

for Carboxylic Acids



A5570 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a bromomethyl group, and easily reacts with a carboxyl group to form the corresponding ester in the presence of a base. The resultant ester is stable enough to reach the detector without any decomposition under reversed phase HPLC. Furthermore, it has a characteristic fluorescence based on a coumarin skeleton, thus an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 340 nm and 425 nm, respectively.



Application examples:

[Fatty acids] [1)]

Dissolve 0.01 g of the sample in 0.1 mL of acetone. The solution is neutralized by the addition of 10% KOH / methanol. To the resultant solution, add an acetone solution with an excess amount of labeling reagent 1, 18-crown 6-ether, and potassium carbonate. Close the cap of the reaction vessel and incubate the mixture at 70 °C for 30 min. Cool to room temperature and use it as an HPLC sample solution.

[Others]

Prostaglandins[1)], bile acids[1)], proteins[2)], nucleic acids[3)]

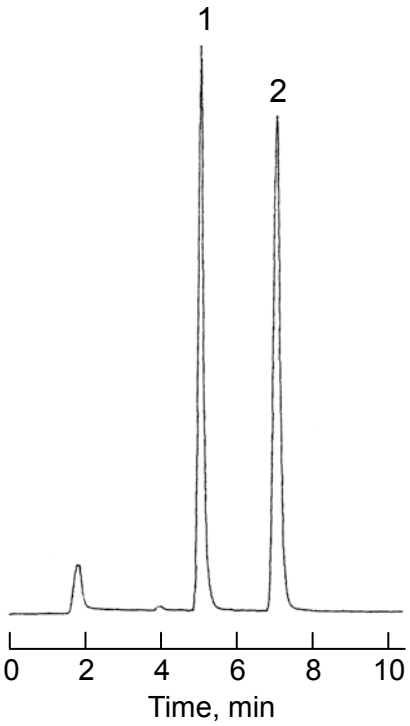
A5570	**4-Bromomethyl-6,7-dimethoxycoumarin**	100 mg	1 g

References

1) a) R. Farinotti, Ph. Siard, J. Bourson, S. Kirkiacharian, B. Valeur, G. Mahuzier, *J. Chromatogr.* **1983**, *269*, 81.
   b) Y. Amet, F. Berthou, J. F. Menez, *J. Chromatogr. B* **1996**, *681*, 233.
   c) A. J. J. M. Coenen, M. J. G. Kerkhoff, R. M. Heringa, Sj. van der Wal, *J. Chromatogr.* **1992**, *593*, 243.
2) a) T. Hiratsuka, *J. Biochem.* **1987**, *101*, 1457.
   b) H. I. Stefanova, J. M. East, M. G. Gore, A. G. Lee, *Biochemistry* **1992**, *31*, 6023.
3) a) S. Yoshida, T. Adachi, S. Hirose, *J. Chromatogr.* **1988**, *430*, 156.
   b) S. Yoshida, T. Adachi, S. Hirose, *Microchem. J.* **1989**, *39*, 351.

AZ-571

Chromatogram of fatty acids as 6,7-Dimethoxycoumarin 4-methyl esters

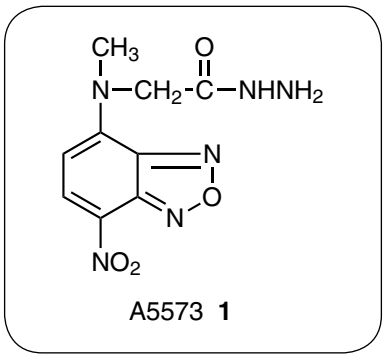


Column	: Kaseisorb LC ODS Super 4.6 mmI.D.×150 mm		
Mobile Phase	: CH_3CN		
Detector	: Fluorescence	λex	340 nm
		λem	425 nm
Temperature	: Ambient		
Flow Rate	: 1 mL / min		

1. Linolic Acid
2. Oleic Acid

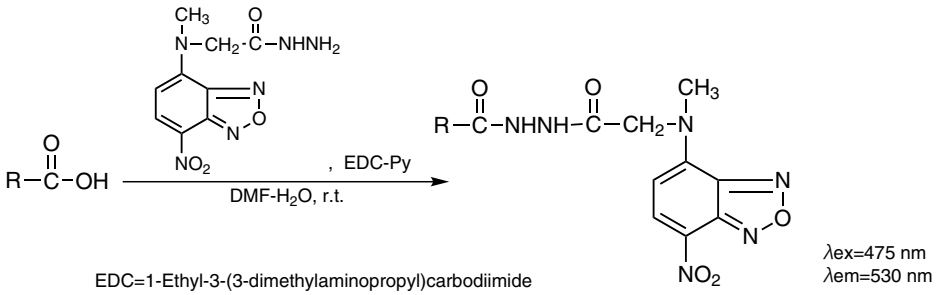
HPLC Labeling Reagent

for Carboxylic Acids



A5573 **1**

The compound **1**, an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a hydrazino group, easily reacts with a carboxyl group to form the corresponding carbohydrazide in the presence of a condensing agent. The resultant carbohydrazide is stable for at least one week at 4 ºC. The carbohydrazide derivatives can be analyzed by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 475 nm and 530 nm, respectively. [The detection limit = 2~4 fmol (S/N = 3)]



EDC=1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide

Application example:

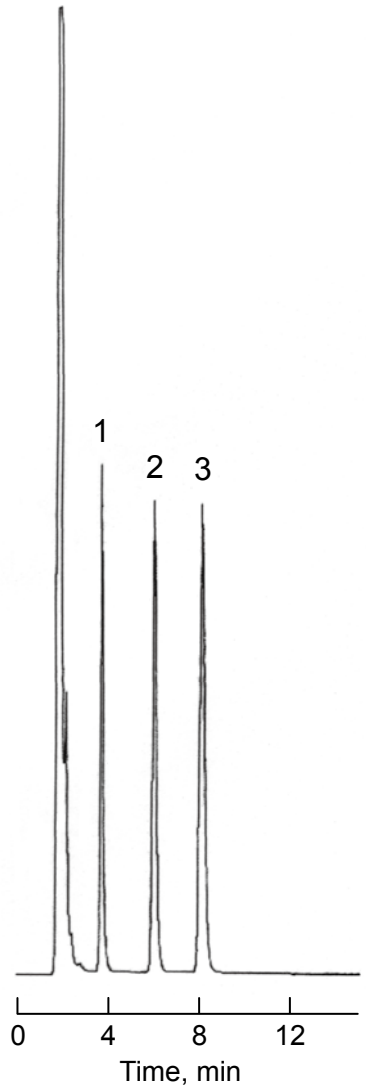
Add 50 μL of carboxylic acid / DMF solution, 50 μL of 1.0 M EDC aqueous solution, 50 μL of 20% pyridine aqueous solution and 20 mM HPLC labeling reagent **1** / DMF solution to a vessel, and incubate the mixture at room temperature for 2 h. Dilute this reactant mixture 10x with the mobile phase solution, and use 1 μL of this diluted solution as an HPLC sample solution.

A5573	**NBD-CO-Hz** **[=4-(*N*-Hydrazinocarbonylmethyl-*N*-methylamino)-7-nitro-2,1,3-benzoxadiazole]**	100 mg

Reference

1) T. Santa, A. Takeda, S. Uchiyama, T. Fukushima, H. Homma, S. Suzuki, H. Yokosu, C. K. Lim, K. Imai, *J. Pharm. Biomed. Anal.* **1998**, *17*, 1065.

Chromatogram of non-steroidal anti-inflammatory drugs as NBD-CO-Hz derivatives

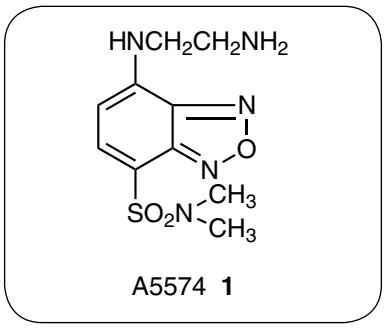


Column : Kaseisorb LC ODS 2000
4.6 mmI.D.×150 mm
Mobile Phase : CH_3OH / H_2O = 70 / 30
Detector : Fluorescence λex 475 nm
λem 530 nm
Temperature : Ambient
Flow Rate : 1.0 mL / min

1. Ketoprofen
2. Flurbiprofen
3. Ibuprofen

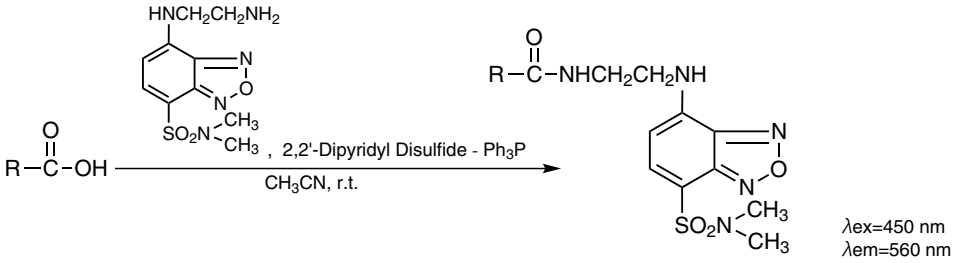
HPLC Labeling Reagent

for Carboxylic Acids



A5574 **1**

The compound **1**, an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an amino group, easily reacts with a carboxyl group to form the corresponding amide in the presence of a condensing agent. The resultant amide is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 560 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference from contaminants. Short-chain fatty acids are detectable and determinable reproducibly with a detection limit on the order of fmol. A highly sensitive detection can be done by using chemiluminescence.



Application example:[2)]

Add 50 μL of mixed fatty acid / diethyl ether solution, 50 μL of 50 mM labeling reagent **1** / acetonitrile solution, 50 μL of triphenylphosphine / acetonitrile solution and 50 μL of 2,2'- dipyridyl disulfide / acetonitrile solution to a vessel. This mixture is kept in the dark at room temperature. Dilute this reactant mixture 100x by acetonitrile, and use 10 μL of this diluted solution as an HPLC sample solution.

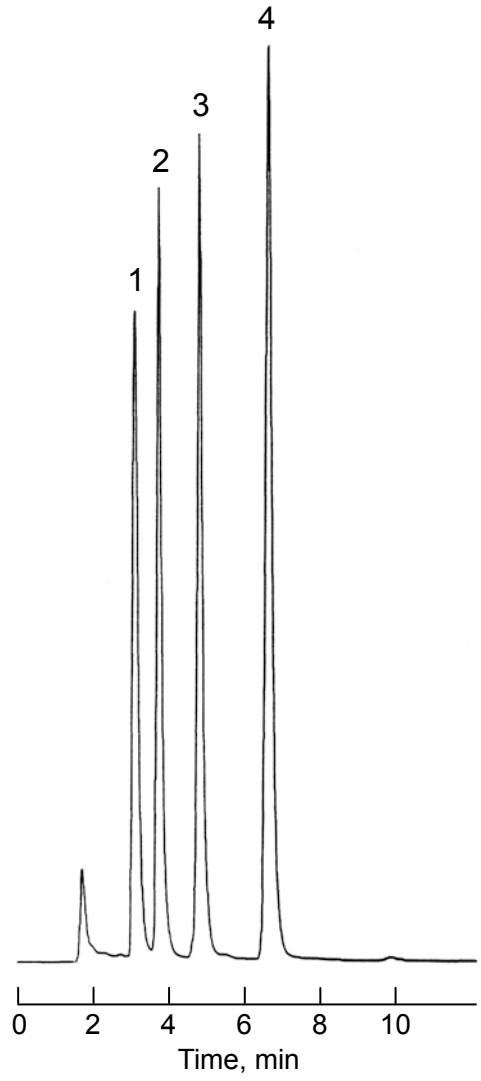
A5574	**DBD-ED** **[=4-(*N,N*-Dimethylaminosulfonyl)-7-(2-aminoethylamino)-2,1,3-benzoxadiazole]**	100 mg

References

1) Tokyo Kasei Kogyo, Jpn. Kokai Tokkyo Koho 98 218871, **1998**.
2) P. Prados, T. Fukushima, T. Santa, H. Homma, M. Tsunoda, S. Al-Kindy, S. Mori, H. Yokosu, K. Imai, *Anal. Chim. Acta* **1997**, *344*, 227.

AZ-575

Chromatogram of fatty acids as DBD-ED derivatives

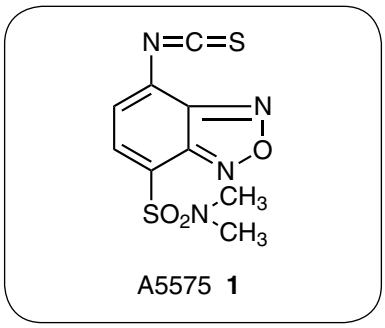


Column : Kaseisorb LC ODS 2000
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 95 / 5
Detector : Fluorescence λex 450 nm
λem 560 nm
Temperature : 40 ºC
Flow Rate : 1.0 mL / min

1. Linolenic Acid
2. Linolic Acid
3. Oleic Acid
4. Stearic Acid

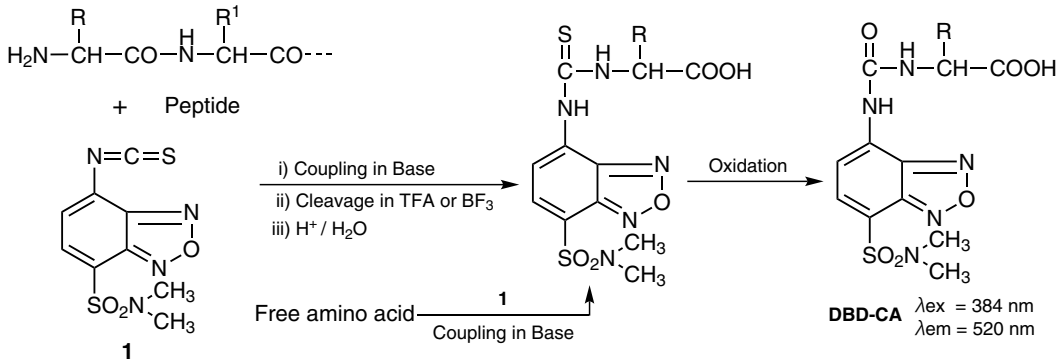
HPLC Labeling Reagent

for Amines



A5575 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and an isothiocyano group, and easily reacts with an amino group to form the corresponding thiourea. The resultant thiourea is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 384 nm and 520 nm, respectively. The detection limit for its quantity is an order of sub-picomol (S/N = 3). **1** itself does not fluoresce but shows an excellent stability in forms of both crystal and solution, and its derivatives are also stable. This compound can be used for amino acid sequence analysis (Edman Degradation) by binding with the *N*-terminal amino acid of peptides or proteins, followed by acid treatment.



1

AZ-576

Application example:

[Method by Manual Edman Degradation]

Peptide (insulin Chain B 500 pmol)

- Dissolve in 20 μL of 50% pyridine / H_2O.
- Add 5 μL of 1% triethylamine / CH_3CN and 10 μL of 20 mM HPLC labeling reagent **1** / pyridine, and react the mixture at 50 ºC for 15 min under the atmosphere of inert gas.
- After cooling to room temperature, wash the reactant solution 3 times with 200 μL of heptane / dichlorormethane (6/4).
- Dry the washed solution at 50 ºC for 15 min by using a centrifugation evaporator.
- Add 30 μL of 1% BF_3•Et_2O / CH_3CN to the mixture and incubate the mixture at 50 ºC for 5 min.
- Further dry the reactant solution under nitrogen gas.
- Add 20 μL of H_2O, and then extract 2 times with 100 μL of benzene / AcOEt (1/4).

(Aqueous phase)
A peptide will be eluted out.

(Organic phase)

- Dry the extracted organic phase under nitrogen gas.
- Dissolve the mixture in 2 μL of CH_3CN.
- Add 8 μL of 0.4 M HCl and hydrolyze the mixture at 50 ºC for 5 min.
- Treat the reactant with 5 μL of 4 M HCl and 0.5 M $NaNO_2$ at room temperature for 10 min and oxidize it.
- Neutralize the reactant with 23 μL of 1 M $NaNO_2$, and remove an excessive oxidant by adding 20 μL of 0.15 M methionine.

Use 20 μL of this solution as an HPLC sample solution.

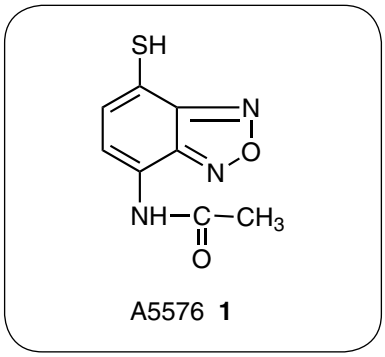
A5575	**DBD-NCS** **[=4-(*N,N*-Dimethylaminosulfonyl)-7-isothiocyanato-2,1,3-benzoxadiazole]**	100 mg

References

1) Y. Huang, H. Matsunaga, A. Toriba, T. Santa, T. Fukushima, K. Imai, *Anal. Biochem.* **1999**, *270*, 257.
2) H. Matsunaga, T. Santa, K. Hagiwara, H. Homma, K. Imai, S. Uzu, K. Nakashima, S. Akiyama, *Anal. Chem.* **1995**, *67*, 4276.
3) K. Imai, S. Uzu, K. Nakashima, S. Akiyama, *Biomed. Chromatogr.* **1993**, *7*, 56.

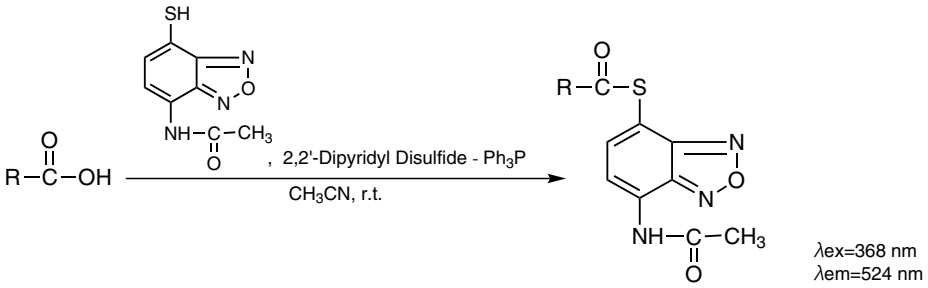
HPLC Labeling Reagent

for Carboxylic Acids



A5576 **1**

The compound **1**, an HPLC fluorescence labeling reagent, which has a 2,1,3-benzoxadiazole skeleton and a mercapto group, easily reacts with a carboxyl group to form the corresponding thioester. **1** itself fluoresces very little, but the thioester derivatives fluoresce highly. The resultant thioester is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 368 nm and 524 nm, respectively. [The detection limit = 10~20 fmol (S/N = 3)]



Application example:

Add 20 μL of mixed fatty acid / acetonitrile solution, 20 μL of 20 mM labeling reagent **1** / dichloromethane solution, 20 μL of triphenylphosphine / acetonitrile solution and 20 μL of 2,2'- dipyridyl disulfide / acetonitrile solution to a 500 μL vessel, and the mixture is left at room temperature for 15 min. Dilute this reactant mixture with 20 μL of acetonitrile, and use 1 μL of this diluted solution as an HPLC sample solution.

A5576	**AABD-SH (=4-Acetamido-7-mercapto-2,1,3-benzoxadiazole)**	100 mg

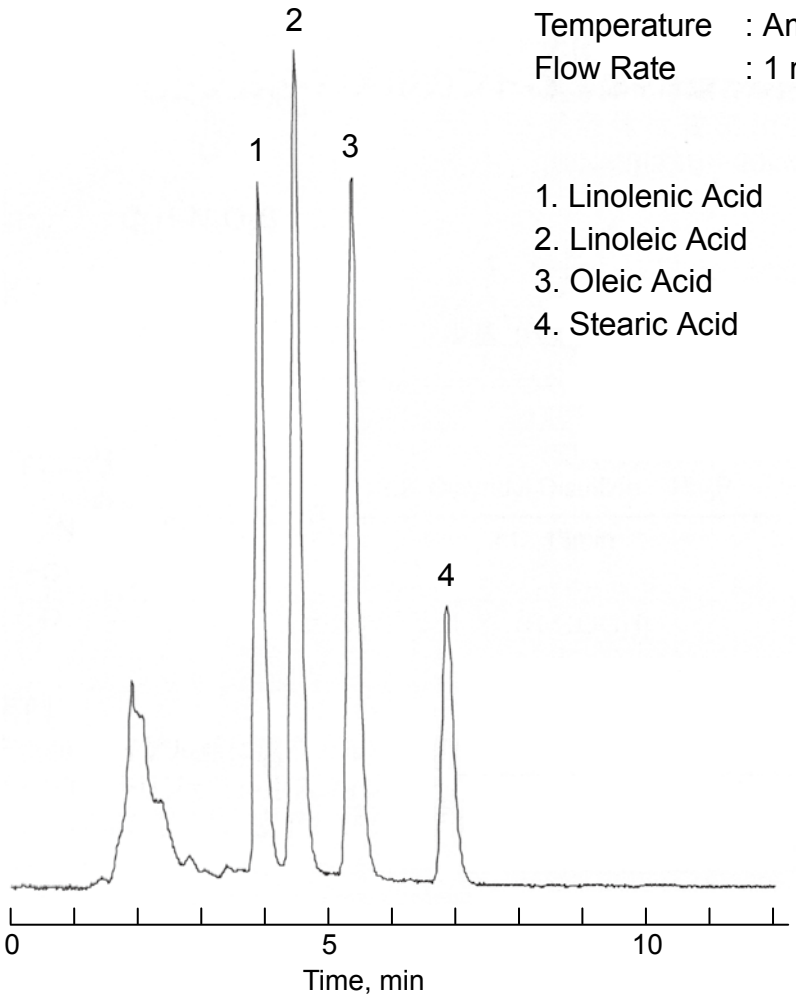
Reference

1) T. Santa, T. Okamoto, S. Uchiyama, K. Mitsuhashi, K. Imai, *Analyst* **1999**, *124*, 1689.

AZ-577

Chromatogram of fatty acids as AABD-thio esters

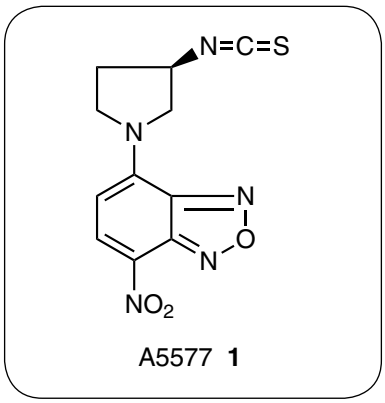
Column : Kaseisorb LC ODS 2000
4.6 mmI.D.×150 mm
Mobile Phase : CH_3OH
Detector : Fluorescence λex 368 nm
λem 524 nm
Temperature : Ambient
Flow Rate : 1 mL / min



1. Linolenic Acid
2. Linoleic Acid
3. Oleic Acid
4. Stearic Acid

HPLC Labeling Reagent

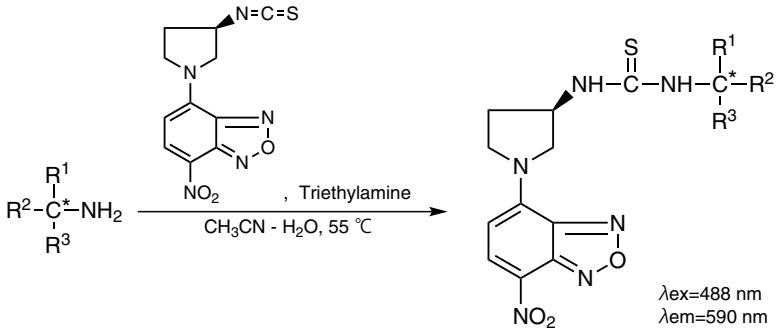
for Chiral Amines



A5577 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino groups, which are directly linked to an asymmetric carbon atom, and produces diastereomers of thiourea. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 488 nm and 590 nm, respectively.

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change the elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.



Application example:

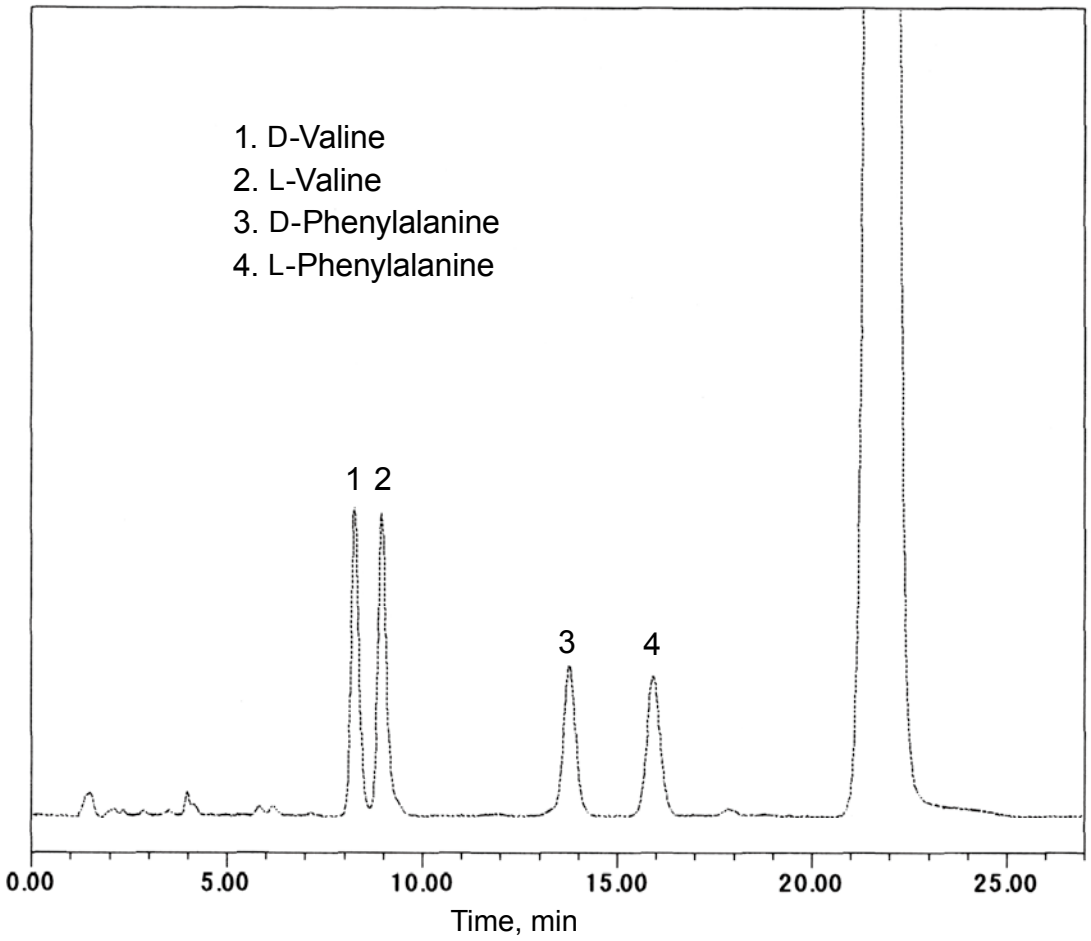
Add 10 μL of 5 mM HPLC labeling reagent 1 / acetonitrile solution in 10 μL of 1 mM amine / acetonitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel. Close the cap of the reaction vessel and incubate the mixture at 55 ºC for 10 min. Then, add 480 μL of a mixture solution of 1 M acetic acid and acetonitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 μL of this diluted solution as an HPLC sample solution.

A5577 **(*R*)-(-)-NBD-Py-NCS** 100 mg
[=(*R*)-(-)-4-(3-Isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole]

AZ-578

Chromatogram of amino acids as (*R*)-(-)-NBD-Py-NCS derivatives

Column : Kaseisorb LC ODS 2000
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 40 / 60
containing 0.05% TFA
Detector : Fluorescence λex 488 nm
λem 590 nm
Temperature : 30 ºC
Flow Rate : 1.0 mL / min

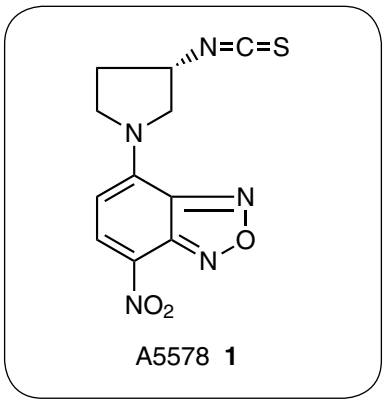


References

1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.

HPLC Labeling Reagent

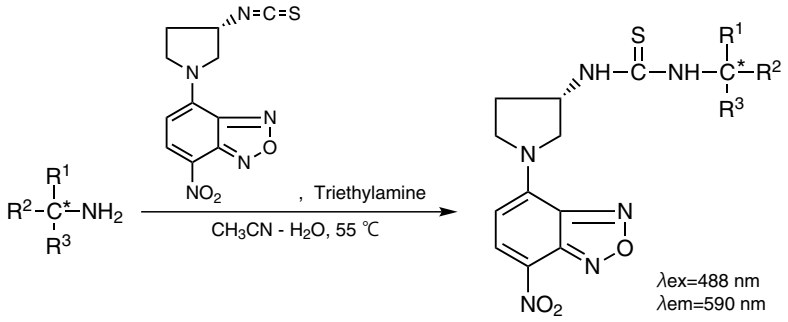
for Chiral Amines



A5578 **1**

The compound **1** is an HPLC fluorescence labeling reagent for optical purity determination. This compound easily reacts with amino groups, which are directly linked to an asymmetric carbon atom, and produces diastereomers of thiourea. These diasteromers can be separated by reversed phase HPLC, and an excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 488 nm and 590 nm, respectively.

Since both (*R*)- and (*S*)-isomers of the derivatization reagents are commercially available on the market, it is possible to change the elution order of the derivatized diastereomers by selecting either enantiomer of the derivatization reagent. Thus, an enantiomer of the amino-compound, whose existing quantity is very small, can be eluted out first and quantified with high precision. Moreover, there are reports for the application of these isomers to Edman Degradation.



Application example:

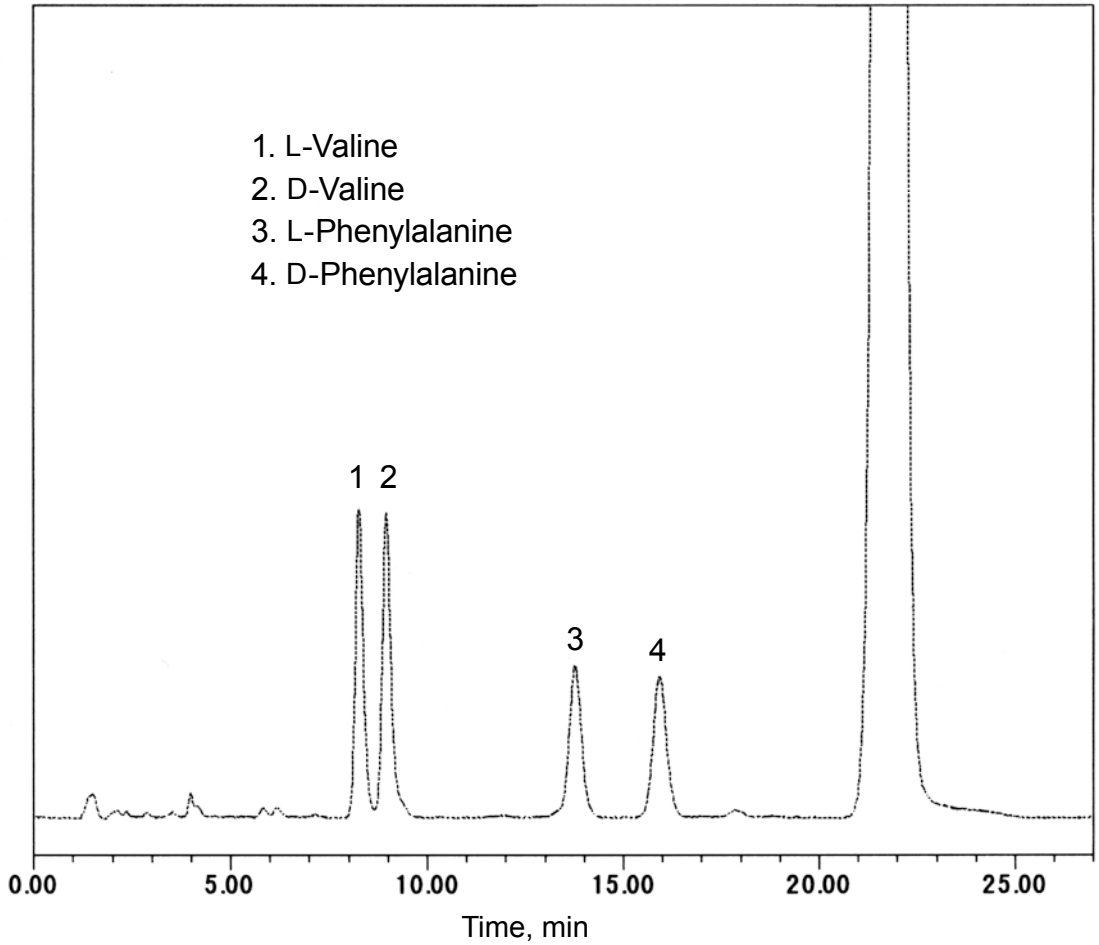
Add 10 μL of 5 mM HPLC labeling reagent **1** / acetonitrile solution in 10 μL solution of 1 mM amine / acetonitrile-H_2O (1:1) solution (containing 2% triethylamine) to a vessel. Close the cap of the reaction vessel and incubate the mixture at 55 ºC for 10 min. Then, add 480 μL of a mixture solution of 1 M acetic acid and acetonitrile-H_2O (1:1) solution, and dilute this reactant mixture 10x by acetonitrile. Use 5 μL of this diluted solution as an HPLC sample solution.

A5578	**(*S*)-(+)-NBD-Py-NCS** **[=(*S*)-(+)-4-(3-Isothiocyanatopyrrolidin-1-yl)-7-nitro-2,1,3-benzoxadiazole]**	100 mg

AZ-579

Chromatogram of amino acids as (*S*)-(+)-NBD-Py-NCS derivatives

Column : Kaseisorb LC ODS 2000
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 40 / 60
containing 0.05% TFA
Detector : Fluorescence λex 488 nm
λem 590 nm
Temperature : 30 ºC
Flow Rate : 1.0 mL / min

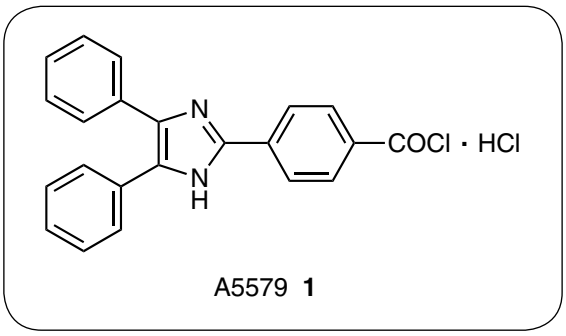


References

1) T. Toyo'oka, Y.-M. Liu, *Analyst* **1995**, *120*, 385.
2) T. Toyo'oka, Y.-M. Liu, *J. Chromatogr. A* **1995**, *689*, 23.
3) T. Toyo'oka, Y.-M. Liu, *Chromatographia* **1995**, *40*, 645.
4) Y.-M. Liu, J.-R. Miao, T. Toyo'oka, *Anal. Chim. Acta* **1995**, *314*, 169.

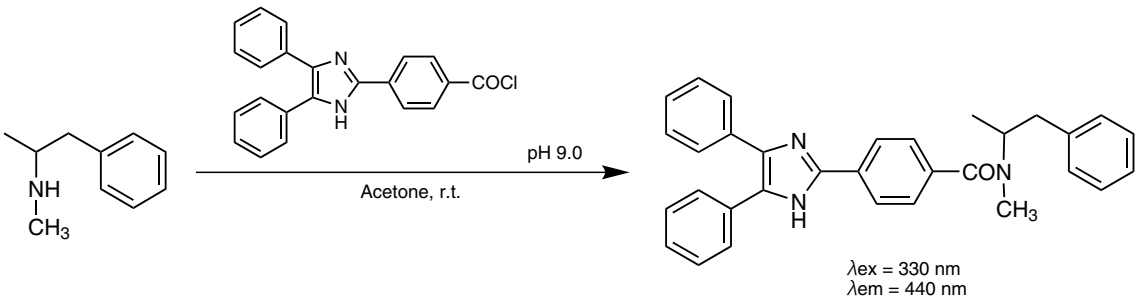
HPLC Labeling Reagent

for Amines and Alcohols



A5579 **1**

The compound **1** is an HPLC fluorescence labeling reagent, which easily reacts with amino groups and hydroxyl groups to form the corresponding amides and esters, respectively. These derivatives are stable for at least 24 h at room temperature, and can reach the detector without any decomposition under reversed phase HPLC. Each derivative can be separated with ODS columns, and the detection limits (S/N = 3) are from 0.6 to 5.2 fmol / 5 μL injection[1)]. **1** is used for the quantitative analysis of methamphetamine and the derivatives in hair[3)], which is known to preserve drugs for a long term, as well as in urine[1,2)].



Application example:

[Quantitative analysis for methamphetamine analogs] [2)]

10 μL of urine collected from a methamphetamine addict and 10 μL of acetic acid are put into an amber-glass vial and dried under a flow of nitrogen. 10 μL of carbonate buffer solution and 190 μL of 100 μM labeling reagent **1** / acetone solution are added to the residue, reacted at room temperature for 10 min. Use it as an HPLC sample solution.

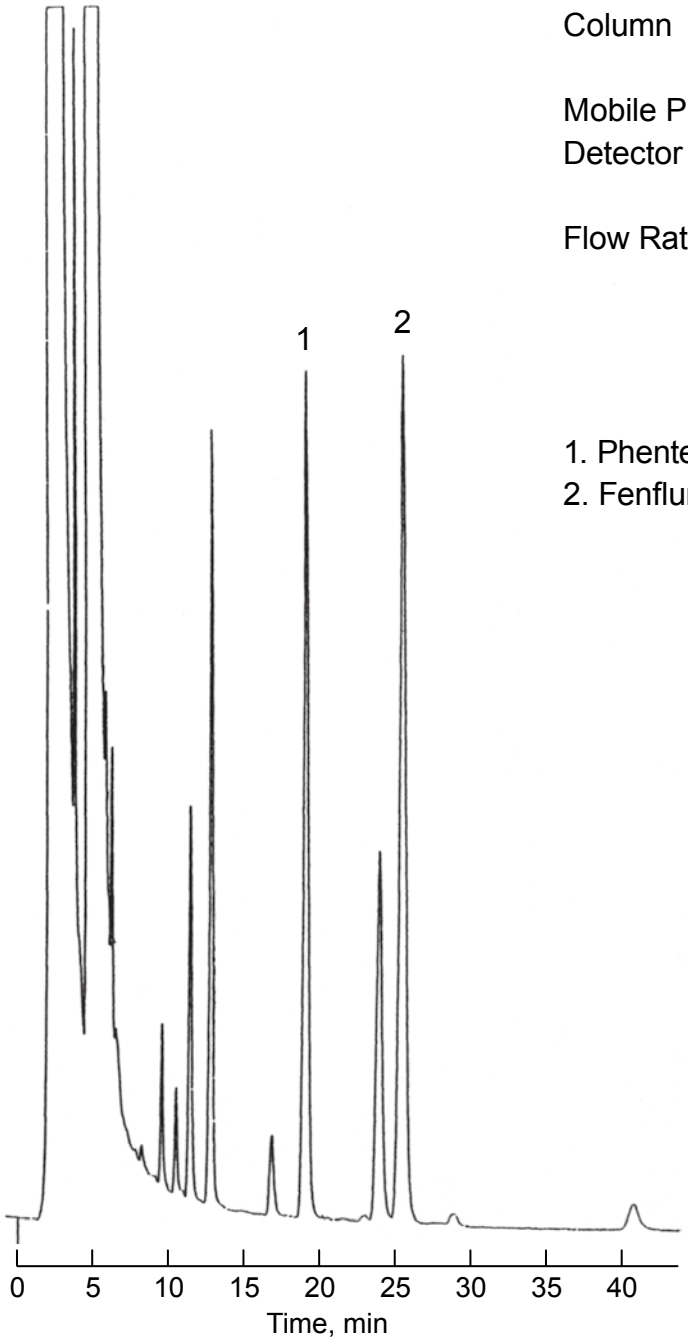
A5579	**4-(4,5-Diphenyl-1*H*-imidazol-2-yl)benzoyl Chloride Hydrochloride**	100 mg

References

1) O. Al-Dirbashi, J. Qvarnstrom, K. Irgum, K. Nakashima, *J. Chromatogr. B* **1998**, *712*, 105.
2) O. Al-Dirbashi, N. Kuroda, F. Menichini, S. Noda, M. Minemoto, K. Nakashima, *Analyst* **1998**, *123*, 2333.
3) O. Y. Al-Dirbashi, N. Kuroda, M. Wada, M. Takahashi, K. Nakashima, *Biomed. Chromatogr.* **2000**, *14*, 293.
4) K. Nakashima, S. Kinoshita, M. Wada, N. Kuroda, W. R. G. Baeyens, *Analyst* **1998**, *123*, 2281.
5) M. Wada, S. Kinoshita, Y. Itayama, N. Kuroda, K. Nakashima, *J. Chromatogr. B* **1999**, *721*, 179.

AZ-580

Chromatogram of amines as DIB derivatives

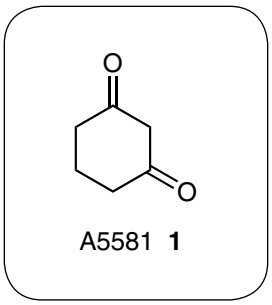


Column : Daisopak SP-120-5-ODS-BP
4.6 mmI.D.×250 mm
Mobile Phase : CH_3CN / H_2O = 65 / 35
Detector : Fluorescence λex 330 nm
λem 440 nm
Flow Rate : 1.0 mL / min

1. Phentermine
2. Fenfluramine

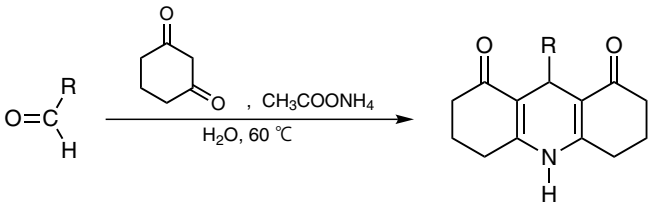
HPLC Labeling Reagent

for Carbonyl Compounds



A5581 **1**

The compound **1** is an HPLC fluorescence labeling reagent, and can easily react with a carbonyl groups to form the corresponding decahydroacridine-1,8-dion (DHA) derivative. The resultant derivative is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection analysis at the excitation and emission wavelengths of 366 nm and 440 nm, respectively.



Application example:

[Aliphatic aldehydes] [1,2)]

5 mL of acetic acid and 10 g of ammonium acetate are dissolved in distilled water. Then 0.25 g of labeling reagent **1** is added to the solution and shaken to prepare the derivatization reagent solution. 2 mL of this solution is added to 1 mL of aqueous solution (ethanol solution, in the case of long-chain aldehydes) containing 10~30 ng of an aliphatic aldehyde, and incubate at 60 ºC for 30 min. After cooling, use 1 μL of this solution as an HPLC sample.

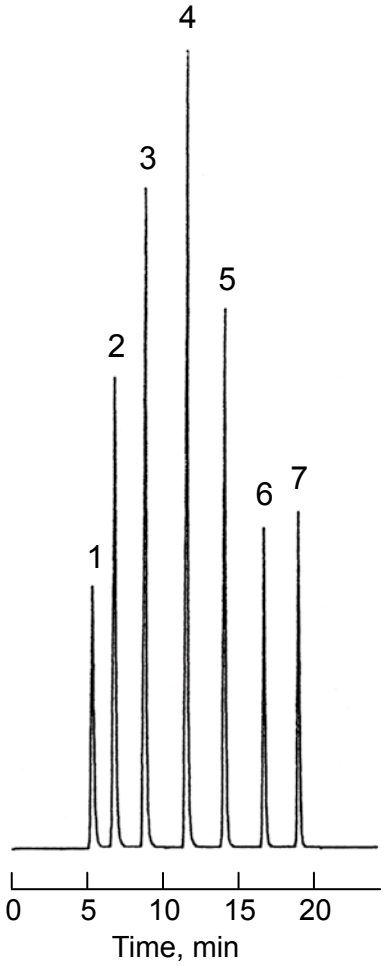
A5581	**1,3-Cyclohexanedione**	5 g

References

1) W. L. Stahovec, K. Mopper, *J. Chromatogr.* **1984**, *298*, 399.
2) Y. Suzuki, *Bunseki Kagaku* **1985**, *34*, 314.

AZ-582

Chromatogram of aldehydes as DHA derivatives

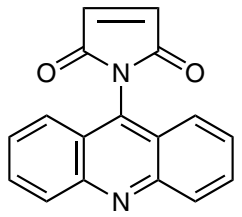


Column : Kaseisorb LC ODS-100-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3OH / H_2O =
40 / 60 → 90 / 10
20 min. linear gradient
Detector : Fluorescence λex 366 nm
λem 440 nm
Flow Rate : 1 mL / min

1. Formaldehyde
2. Acetaldehyde
3. Propionaldehyde
4. Butyraldehyde
5. Valeraldehyde
6. Hexylaldehyde
7. Heptylaldehyde

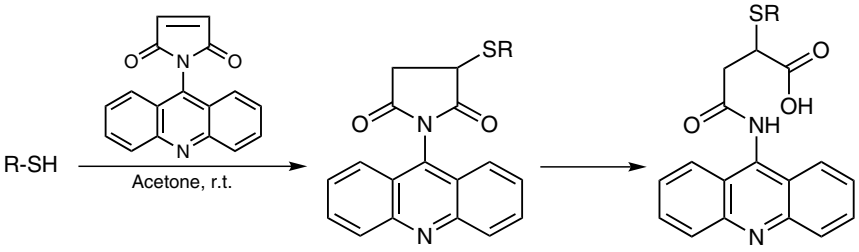
HPLC Labeling Reagent

for Thiols



A5591 **1**

The compound **1** is an HPLC fluorescence labeling reagent, and can easily react with a mercapto group at room temperature. The resultant sulfide is stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection analysis at the excitation and emission wavelengths of 355 nm and 465 nm, respectively.



Application example:

[Thiols] [1~5)]

0.4 mL of 30% NaOH solution and 1 mL of 0.2 M boric acid buffer solution (pH 8.8) are added to 2 mL of 1 mM sample solution in water. To this solution, 0.5 mL of 10 mM labeling reagent **1** / acetone solution is added and shaken, and reacted at room temperature for 30 min to use it as a HPLC sample.

A5591	**NAM [=*N*-(9-Acridinyl)maleimide]**	50 mg	100 mg

References

1) Y. Nara, K. Tujimura, *Bunseki Kagaku* **1973**, *22*, 451.
2) Y. Nara, K. Tujimura, *Agric. Biol. Chem.* **1978**, *42*, 793.
3) H. Takahashi, Y. Nara, K. Tujimura, *Agric. Biol. Chem.* **1979**, *43*, 1439.
4) H. Takahashi, Y. Nara, K. Tujimura, *Agric. Biol. Chem.* **1976**, *40*, 2493.
5) H. Takahashi, T. Yoshida, H. Meguro, *Bunseki Kagaku* **1981**, *30*, 339.

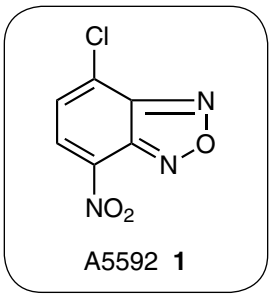
Chromatogram of thiols as NAM derivatives

Column : Kaseisorb LC ODS-300-5
4.6 mmI.D.×150 mm
Mobile Phase : 0.05 M Na_2HPO_4 / CH_3CN
=89 / 11 (pH 7.5)
Detector : Fluorescence λex 355 nm
λem 465 nm
Flow Rate : 1 mL / min

1. *N*-Acetyl-L-cysteine
2. 2-Mercaptoethanol

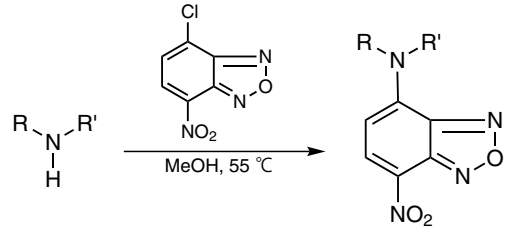
HPLC Labeling Reagent

for Amines and Thiols



A5592 **1**

The compound **1** which is an HPLC fluorescence labeling reagent having a 2,1,3-benzoxadiazole skeleton, can easily react with a secondary amine and thiol. The resultant derivative is stable enough to reach the detector without any decomposition under general reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection analysis at the excitation and emission wavelengths of 460 nm and 535 nm, respectively.



Application examples:

[Alkylamines] [1)]

To 25~500 μL of a methanol solution containing an amine (1~20 μg), 4~8 eq. excess amount of 0.05% labeling reagent **1** / methanol solution is added. After adding 50~100 μL of 0.1 M $NaHCO_3$, incubate at 55 ºC for 1~5 h. After cooling the reaction mixture to room temperature, use it as an HPLC sample.

[Others]

TLC and HPLC of *N*-methylcarbamates, *N*,*N*-dimethylcarbamates in agrichemicals[2,3)]
Hydrolyze the carbamates to label the amine derivatives.

TLC of amphetamines in urine[4,5)], HPLC of prolines (precolumn derivatization method)[6)]

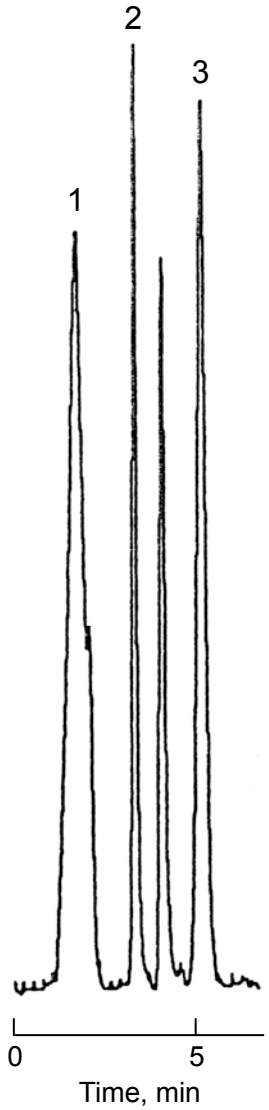
A5592	**NBD-Cl (= 4-Chloro-7-nitro-2,1,3-benzoxadiazole)**	1 g	5 g

References

1) H.-J. Klimisch, L. Stadler, *J. Chromatogr.* **1974**, *90*, 141.
2) J. F. Lawrence, R. W. Frei, *Anal. Chem.* **1972**, *44*, 2046.
3) R. W. Frei, J. F. Lawrence, *J. Assoc. Off. Anal. Chem.* **1972**, *55*, 1259.
4) J. Monforte, R. J. Bath, I. Sunshine, *Clin. Chem.* **1972**, *18*, 1329.
5) F. van Hoof, A. Heyndrickx, *Anal. Chem.* **1974**, *46*, 286.
6) J. H. Wolfram, *J. Chromatogr.* **1977**, *132*, 37.
7) Y. Nishikawa, K. Kuwata, *Anal. Chem.* **1985**, *57*, 1864.

AZ-593

Chromatogram of alkylamines as NBD derivatives

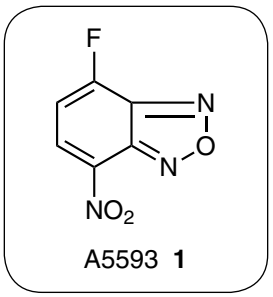


Column : Kaseisorb LC ODS-300-5
4.6 mmI.D.×150 mm
Mobile Phase : CH_3CN / H_2O = 45 / 55
Detector : Fluorescence λex 460 nm
λem 535 nm
Flow Rate : 1 mL / min

1. Propylamine
2. Butylamine
3. Amylamine

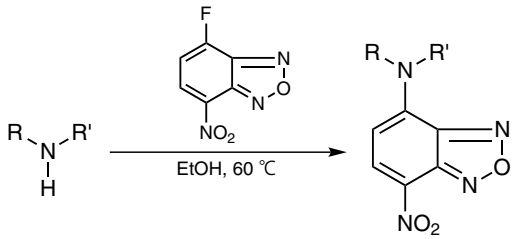
HPLC Labeling Reagent

for Amines and Thiols



A5593 **1**

The compound **1** which is an HPLC fluorescence labeling reagent having a 2,1,3-benzoxadiazole skeleton, can easily react with amino or mercapto groups to form the corresponding derivatives. **1** itself does not fluoresce, and its ethanol solution is relatively stable for a week in a refrigerator. The derivatives can reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 470 nm and 530 nm, respectively. Since their excitation and fluorescence wavelengths are at longer wavelengths, detection has less interference by contaminants. Thus, further highly sensitive detection can be done by using laser induced fluorescence detector. When the reagent is hydrolyzed (NBD-OH), its fluorescence can be erased under an acidic condition. Therefore, this hydrolyzed reagent can be used as a post column reaction reagent[5,7].



Application example:

[Amino acids] [2,3]

To 10 μL of 50 μM amino acid standard solution, add 10 μL of 0.1 M boric acid buffer solution (pH 8.0) and 20 μL of 50 mM labeling reagent **1** in ethanol solution, and incubate the mixture at 60 ºC for 1 min. Immediately cool it with ice bath, and add 460 μL of 5 mM HCl to the reactant solution. Use 10 μL of the solution as an HPLC sample.

A5593	**NBD-F (=4-Fluoro-7-nitro-2,1,3-benzoxadiazole)**	100 mg

References

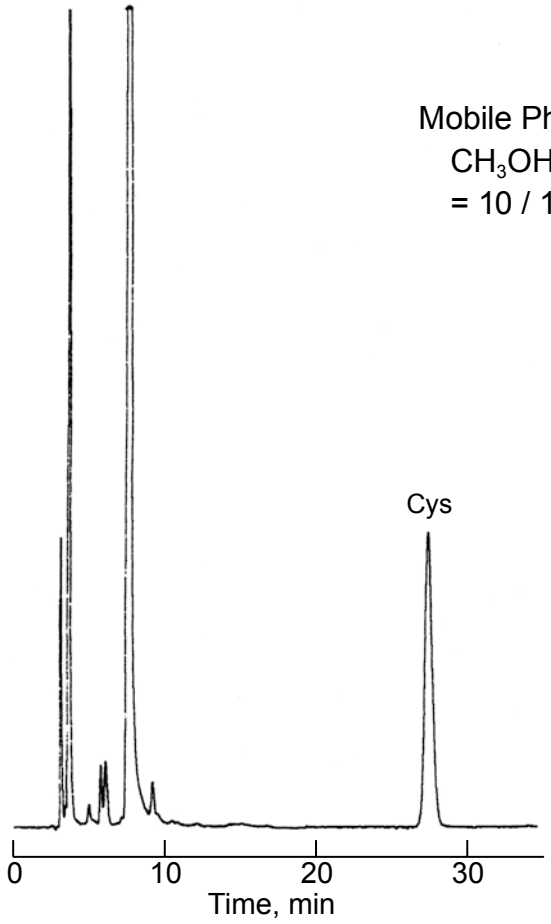
1) K. Imai, Y. Watanabe, *Anal. Chim. Acta* **1981**, *130*, 377.
2) Y. Watanabe, K. Imai, *Anal. Biochem.* **1981**, *116*, 471.
3) Y. Watanabe, K. Imai, *J. Chromatogr.* **1982**, *239*, 723.
4) T. Toyo'oka, Y. Watanabe, K. Imai, *Anal. Chim. Acta* **1983**, *149*, 305.
5) Y. Watanabe, K. Imai, *Anal. Chem.* **1983**, *55*, 1786.
6) Y. Watanabe, K. Imai, *J. Chromatogr.* **1984**, *309*, 279.
7) H. Miyano, T. Toyo'oka, K. Imai, *Anal. Chim. Acta* **1985**, *170*, 81.
8) H. Kotaniguchi, M. Kawakatsu, T. Toyo'oka, K. Imai, *J. Chromatogr.* **1987**, *420*, 141.

AZ-594

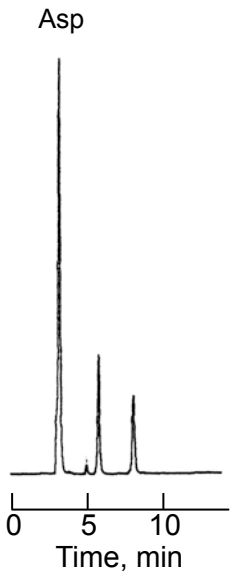
Chromatogram of amino acids as NBD derivatives

Column : Kaseisorb LC ODS Super 4.6 mmI.D.×250 mm
Detector : Fluorescence λex 470 nm λem 530 nm
Temperature : 40 ºC
Flow Rate : 1 mL / min

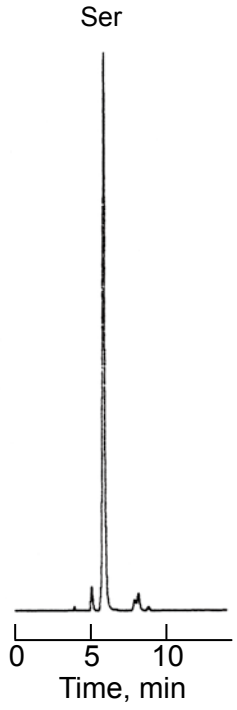
Mobile Phase:
CH_3OH / THF / 0.1 M Phosphate buffer (pH 6.0)
= 20 / 20 / 60



Mobile Phase:
CH_3OH / THF / 0.1 M Phosphate buffer (pH 6.0)
= 10 / 10 / 80

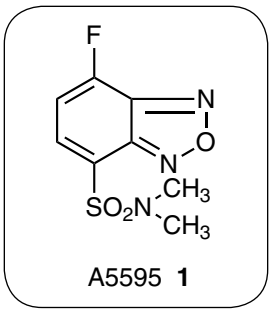


Mobile Phase:
CH_3OH / THF / 0.1 M Phosphate buffer (pH 6.0)
= 10 / 10 / 80



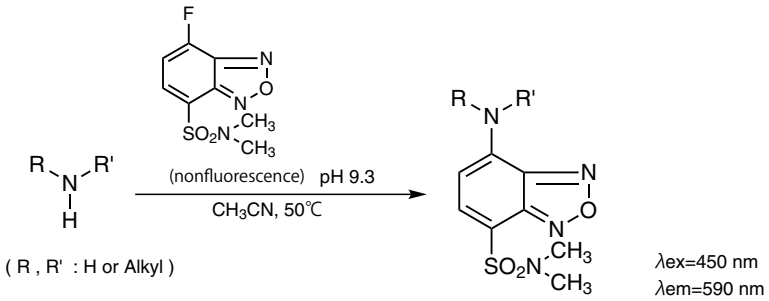
HPLC Labeling Reagent

for Amines and Thiols



A5595 **1**

The compound **1** which is an HPLC fluorescence labeling reagent having a 2,1,3-benzoxadiazole skeleton, can easily react with amino and mercapto groups to form the corresponding derivatives. The derivatives are stable enough to reach the detector without any decomposition under reversed phase HPLC. An excellent chromatogram can be obtained by fluorescence detection at the excitation and emission wavelengths of 450 nm and 590 nm, respectively.



Application example:

[Amino acids]

0.5 mL of 20 mM labeling reagent in acetonitrile is put into an amber-glass vial. To this solution, add 0.5 mL of 0.1 M boric acid buffer solution (pH 9.3, containing 1mM $EDTANa_2$) containing several nmol of an amino acid, and incubate at 50 ºC for 30 min. After cooling the reaction mixture with ice bath, use it as an HPLC sample.

For example, the detection limit (S/N=3) for proline is 0.11 pmol.

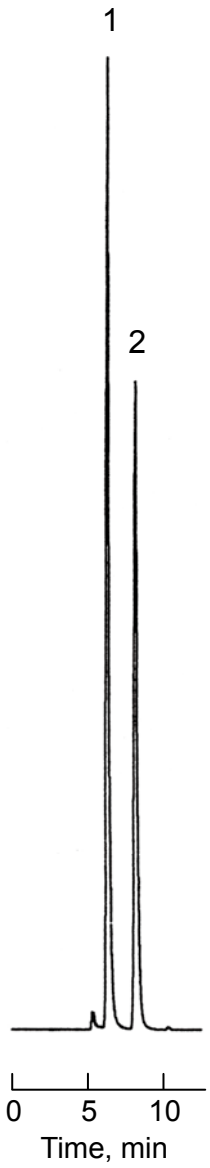
A5595	**DBD-F** **[=4-(*N,N*-Dimethylaminosulfonyl)-7-fluoro-2,1,3-benzoxadiazole]**	100 mg

References

1) T. Toyo'oka, T. Suzuki, Y. Saito, S. Uzu, K. Imai, *Analyst* **1989**, *114*, 413.
2) T. Toyo'oka, T. Suzuki, Y. Saito, S. Uzu, K. Imai, *Analyst* **1989**, *114*, 1233.
3) K. Imai, S. Uzu, T. Toyo'oka, *J. Pharm. Biomed. Anal.* **1989**, *7*, 1395.
4) S. Uzu, K. Imai, K. Nakashima, S. Akiyama, *Analyst* **1991**, *116*, 1353.
5) S. Uzu, K. Imai, K. Nakashima, S. Akiyama, *Biomed. Chromatogr.* **1991**, *5*, 184.

AZ-596

Chromatogram of amino acids as DBD-amino acids

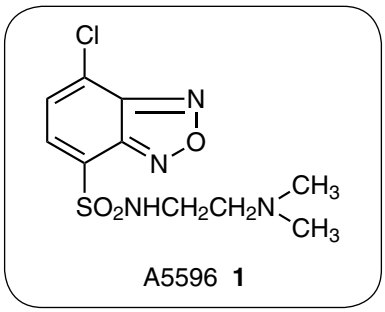


Column : Kaseisorb LC ODS-120-5
4.6 mmI.D.×250 mm
Mobile Phase : CH_3CN / H_2O / CH_3COOH
=50 / 50 / 1
Detector : Fluorescence λex 450 nm
λem 590 nm
Flow Rate : 1 mL / min

1. Valine
2. Leucine

Reagent for Protein Analysis

DAABD-Cl

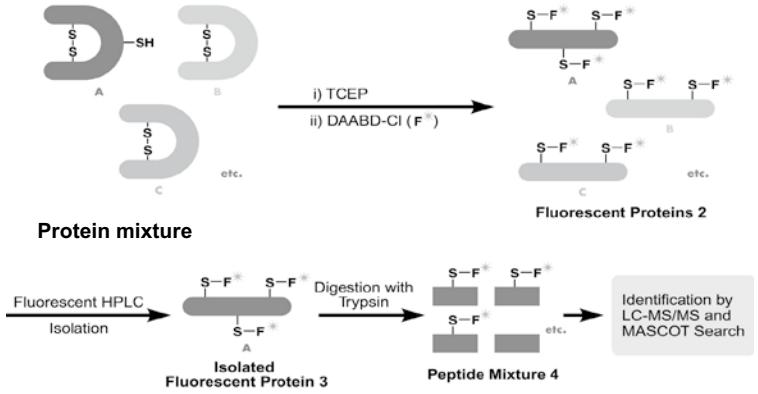


A5596 **1**

The relationship between genes and diseases has been studied extensively since the completion of human genome project in 2003. The direct cause of these diseases is sometimes related to the proteins produced in the human body by the human genome. The study of these proteins, “proteomics”, is very important in order to understand the relationship between genes and diseases.

The general method for protein analysis is isolation of the targeted protein by 2-D gel electrophoresis, followed by digestion with proteases to yield peptide fragment mixtures, which are then analyzed by MS/MS to identify the fragments, from which the isolated protein can then be reconstructed. However several problems still remain with 2-D gel electrophoresis, as extremely acidic, basic, or hydrophobic proteins cannot be fully separated. Furthermore, only the highly skilled experts are able to manage the 2-D gel electrophoresis to obtain reproducible data. For these reasons, new and improved methods for protein analysis have been explored.

Imai and co-workers have developed a new method for protein analysis with use of DAABD-Cl (**1**). This new method can analyze proteins with high precision. Imai and co-workers extracted proteins from breast cancer cells, and the extracted proteins were first reacted with tris(2-carboxyethyl) phosphine in a buffer solution (**2**) in order to reductively cleave the S-S bonds to yield the primary proteins. The resulting SH functional groups of resulting proteins were derivatized by reaction with DAABD-Cl (**1**) to yield fluorescent labeled protein mixtures (2 in Scheme 1). The fluorescent labeled protein mixtures were separated by fluorescence HPLC to obtain fractions consisting of DAABD labeled proteins (Fig. 1). The selected DAABD labeled protein (3 in Scheme 1) was isolated and digested using trypsin to obtain the peptide mixtures (4 in Scheme 1) consisting of DAABD labeled peptides and other peptides. The peptide mixtures were analyzed by LC-MS/MS and the resulting mass spectral data were analyzed to identify the original protein by the MASCOT database system (Scheme. 1).



Scheme 1. Quantification and Identification of Expressed Proteins in cell with DAABD-Cl

A-1094E

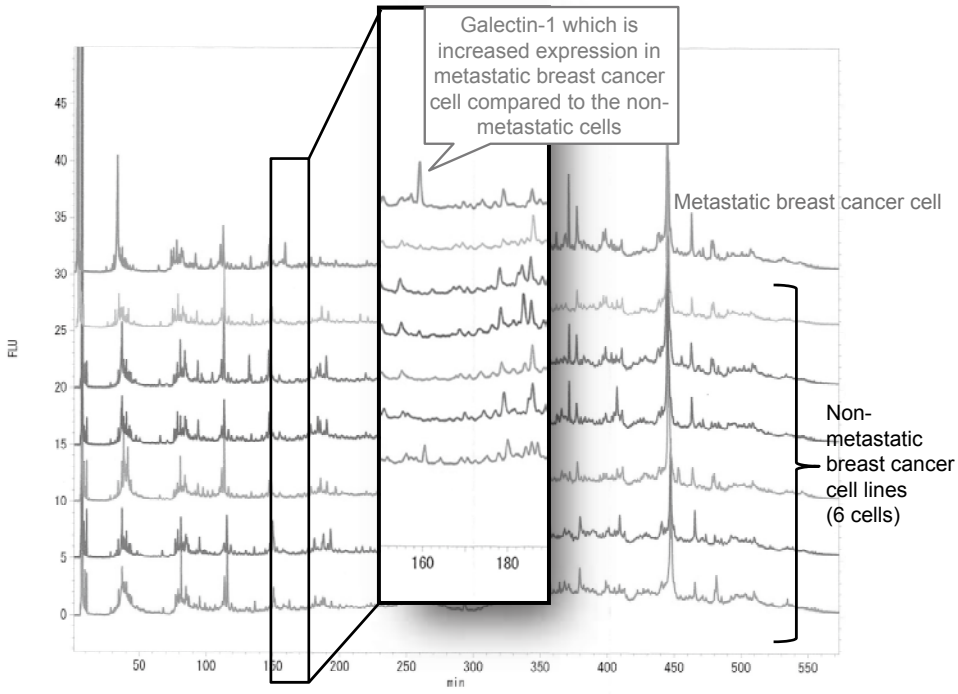


Fig. 1. Chromatograms of the proteins in soluble fraction of breast cancer cells derivatized with DAABD-Cl

The chlorine at 7 position of DAABD-Cl reacts specifically with SH groups. DAABD-Cl itself is non-fluorescent, however the resultant DAABD-derivative is strongly fluorescent, due to the benzoxadiazole skeleton coupled to the SH group. Generally, there are not many S-S bonds and SH group in proteins, and consequently target proteins can be labeled with DAABD-Cl in an efficient manner. Additionally, both excitation and emission wavelengths of DAABD derivatives are long, allowing highly sensitive and selective protein analysis. Furthermore, DAABD-Cl has a dimethylamino group at 4 position, and therefore high intensity cations can be obtained with electron spray ionization during MS analysis. Therefore, extremely small quantities of peptides can be analyzed.

DAABD-Cl is a labeling reagent, which can effectively permit the collection of the target protein through fluorescence HPLC and analysis by MS/MS. This protein analysis reagent that Imai and co-worker have developed allows one to identify a very small amount of protein with good precision. It is expected that this technique (FD-LC-MS/MS method) can be used in many applications, including the identification of abnormal or pathogenic proteins in living organism.

A5596	**DAABD-Cl (1)** **[=4-[2-(Dimethylamino)ethylaminosulfonyl]-7-chloro-2,1,3-benzoxadiazole]**			100 mg
T1656	**Tris(2-carboxyethyl)phosphine Hydrochloride (2)**	1 g	5g	25 g
B2904	**Buffer Solution pH 8.7 (6 mol/L Guanidine Hydrochloride) (3)**			100 mL

References

1) M. Masuda, C. Toriumi, T. Santa, K. Imai, *Anal. Chem.* **2004**, *76*, 728.
2) M. Masuda, H. Saimaru, N. Takamura, K. Imai, *Biomed. Chromatogr.* **2005**, *19*, 556.
3) T. Ichibangase, K. Moriya, K. Koike, K. Imai, *J. Proteome Res.* **2007**, *6*, 2841.
4) H. Asamoto, T. Ichibangase, K. Uchikura, K. Imai, *J. Chromatogr. A* **2008**, *1208*, 147.
5) T. Ichibangase, H. Saimaru, *et al.*, *Biomed. Chromatogr.* **2008**, *22*, 232.
6) K. Imai, T. Ichibangase, R. Saitoh, Y. Hoshikawa, *Biomed. Chromatogr.* **2008**, *22*, 1304.
7) T. Ichibangase, K. Imai, *J. Proteome Res.* **2009**, *8*, 2129.
8) K. Imai, A. Koshiyama, K. Nakata, *Biomed. Chromatogr.* **2011**, *25*, 59.

9) K. Nakata, R. Saitoh, J. Amano, A. Koshiyama, T. Ichibangase, *et al.*, *Cytokine* **2012**, *59*, 317.
10) K. Imai, JP Patent 4558297.
11) *Quantitative Proteome Analysis: Methods and Applications*, ed. by K. Imai, S. L. F. Yau, Pan Stanford Publishing, Singapore, **2013**.
12) K. Nakata, T. Ichibangase, R. Saitoh, M. Ishigai, K. Imai, *Analyst* **2015**, *140*, 71.

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