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Preparation and use of (*S*)-*O*-acetyllactyl chloride (Mosandl's reagent) as a chiral derivatizing agent

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Abstract

(*S*)-*O*-Acetyllactyl chloride is used as a versatile chiral derivatizing agent for the chromatographic determination of the enantiomeric excesses of alcohols or amines. However, some precautions must be taken to avoid its racemization during preparation and use. In addition, the racemic counterpart of this reagent can be used to determine the best analytical separation conditions.

1. Introduction

Enantiodifferentiation and its measurement is a current analytical problem in experiments concerned with enzymatic or microbial transformations: for example, assays for the preparation of enantiomeric secondary alcohols by enzymatic resolution of their racemic esters or by bioreduction of the parent ketones involve quantitative stereochemical determinations which have to combine sensitivity (low amounts of product available in screening experiments), accuracy (exact detection of low amounts of undesired enantiomer) and uncomplicated operation (for use in fast monitoring of the bioconversion). Mainly chromatographic techniques, either directly on a crude biotransformation sample or after a simple derivatization, can fulfill this requirement: using gas or liquid phase chromatography, direct separation on chiral stationary phases (or with chiral mobile phases) or indirect separation after diastereomeric derivatization of the analyte with a proper chiral reagent have been alternatively used. The former is often preferred as it gives an unbiased value of enantiomeric excess, independently of the eventually necessary derivatizing reaction; the latter has to be cautiously controlled at the derivatizing stage in order to avoid distortion of the enantiomeric composition resulting from racemization and/or kinetic factors. On the other hand, the use of chiral stationary phases is generally limited by their relatively narrow specificity and their high cost, while a number of derivatizing agents have been designed for all kinds of molecules,

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and the derivatives thereof obtained can be separated on simple and common chromatographic materials. Therefore, the indirect separation via diastereomeric derivatization is still widely used.

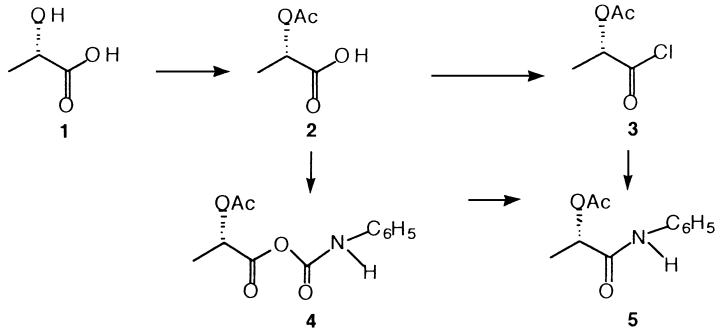
One of the most popular reagents for derivatization was in fact initially developed for the assessment of enantiomeric excess and absolute configuration of alcohols or amines by nuclear magnetic resonance methods and is currently frequently utilized in this way: derivatization with (*S*)-(+)-α-methoxy-α-trifluoromethylphenylacetyl chloride (MTPA chloride) developed by Mosher's group, in preference to *O*-acetylmandelyl chloride or *O*-methylmandelyl chloride, has also been extensively used for separation of diastereomeric esters or amides by high performance liquid chromatography and sometimes by gas chromatography. This reagent which is available in both enantiomeric forms combines other highly desired characteristics, mainly high enantiomeric excess (>99%) and high stability to racemization. However, the use of such a chromophoric derivative, useful for the detection by HPLC, is not so practical for GC separations, owing to the resulting critical decrease in volatility of the derivatized compounds. Furthermore, the increased steric hindrance occurring in the vicinity of the reacting carboxyl group may sometimes prevent a fast and quantitative reaction with hindered secondary alcohols, a prerequisite for the use of a diastereomer-forming reagent.

The simpler chiral acylating agent, (*S*)-*O*-acetyllactyl chloride, derived from enantiomerically pure inexpensive *S*-(+)-lactic acid, has occasionally been utilized for the GC separation of secondary alcohols in the form of α-acetoxypropionylesters.[1–5] More recently, Mosandl[6,7] has emphasized the potential and reliability of such esters (as well as other α-acyloxypropionic esters) in the separation of some chiral secondary alcohols. Since then, this reagent has frequently been used for the measurement of ee of alcohols by GC,[8–17] HPLC[14,18] or NMR,[19] ee of amines[20,21] and for the preparative resolution of alcohols.[22] It has also been used to prepare a chiral phosphonate as a reagent for the determination of enantiomeric excesses of unprotected amino acids.[23] For these purposes it is necessary to make (*S*)-*O*-acetyllactyl chloride available with high enantiomeric excess, and to use it carefully. Indeed, in a few cases, we have observed its epimerization, which leads to an inaccurate measurement of enantiomeric excess. We report here our studies concerning the conditions of preparation and use, and the necessary precautions to be taken, in order that (*S*)-*O*-acetyllactyl chloride should constitute a really versatile and universal acylating derivatizing agent for enantiomer separation.

2. Results and discussion

*2.1. Enantiomeric purity of (*S*)-O-acetyllactyl chloride **3***

Although it is not always necessary to use an enantiopure chiral derivatizing agent,[24] the highest enantiomeric excess is usually preferred. The acid chloride prepared from *S*-(+)-lactic acid **1** with the highest available enantiopurity has to be obtained without any ee decrease. The different steps in the synthesis of the reagent have been analyzed and the enantiomeric excess of intermediates has been measured. For this purpose, it was necessary to accurately determine the ee of (*S*)-*O*-acetyllactic acid **2** and (*S*)-*O*-acetyllactyl chloride **3**. These were measured by gas chromatographic analysis on a chiral column (Chirasilval, Altech) using their *O*-acetyllactic phenyl amide derivative **5** which was obtained as shown in Scheme 1. The reaction of *O*-acetyllactic acid with phenyl isocyanate gave the amide[25] after decarboxylation of the unstable intermediate **4**. This reaction does not require any activation or dehydrating agent and the derivative could be thus obtained in mild conditions (rt, pyridine) without epimerization. On the other hand, the reaction of *O*-acetyllactyl chloride with excess aniline gave the amide **5**. The reaction was carried out at 4°C to avoid epimerization.



Scheme 1.

2.2. *Synthesis of (S)-O-acetyllactyl chloride* **3**

Using our method, we have shown that the commercially available reagent **3** from several sources exhibited an enantiomeric excess lower than 93–94%. We thus preferred to synthesize (*S*)-*O*-acetyllactyl chloride from a solution of *S*-(+)-lactic acid **1** (Fluka), which contains about 10% water, 30% condensation products and less than 1% *R*-(−)-lactic acid (ee >98%).

Acetylation of lactic acid with acetyl chloride is highly exothermic and when the temperature increase was not thoroughly controlled, epimerization of the lactic acid derivative was observed (Table 1, entry 2). When acetyl chloride was slowly added to the lactic acid solution, which was kept below 4°C in an ice-bath (Table 1, entry 1), *O*-acetyllactic acid was obtained in 98% ee. The acid chloride was then obtained without epimerization (97.5–98% ee) by addition of $SOCl_2$ to *O*-acetyllactic acid (Table 1, entry 3) and vacuum distillation after elimination of excess $SOCl_2$ and HCl. At 60°C, in neutral conditions, the acid chloride **3** is stereochemically stable for at least 2 h (Table 1, entry 4). In such conditions, (*S*)-*O*-acetyllactylchloride **3** can be obtained in large amounts and with high enantiomeric purity, and subsequently used extensively and in large excess in derivatization reactions.

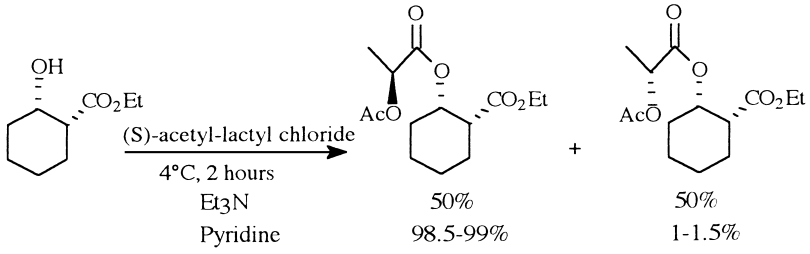
Table 1
Enantiomeric excesses of *O*-acetyllactic acid and *O*-acetyllactic acid chloride prepared in different conditions

	Reagents	Reaction conditions	e.e. %
1	(*S*)-lactic acid e.e.>98 %	1) AcCl, 4°C 2) Phenylisocyanate, Py, RT	98.5
2	(*S*)-lactic acid e.e.>98 %	1) AcCl, RT [a] 2) Phenylisocyanate, Py, RT	50
3	(*S*)-O-acetyllactic acid e.e.= 98.5 %	1) $SOCl_2$ 2) aniline, 4°C	98.5
4	(*S*)-O-acetyllactic chloride e.e.= 98.5 %	1) 60°C, 2 hours 2) aniline, 4°C	97
5	(*S*)-O-acetyllactic chloride e.e.= 98.5 %	aniline, RT [a]	98
6	(*S*)-O-acetyllactic chloride e.e.= 98.5 %	1) Et_3N 1eq, RT, 10 hours 2) aniline, 4°C	60
7	(*S*)-O-acetyllactic chloride e.e.= 98.5 %	1) Py, 60°C 2) aniline, 4°C	94

[a] without temperature control.

2.3. Use of (S)-O-acetyllactic derivatives for ee determination

The derivatization of alcohols was achieved by reaction with (*S*)-*O*-acetyllactyl chloride in diethyl ether in the presence of a nitrogenous base. When the temperature increased during the addition of the base or when the base was triethylamine, epimerization was observed. An example is given in Scheme 2 for the reaction of enantiopure (*cis*) ethyl cyclohexanol-2 carboxylate in the presence of triethylamine or pyridine. The isomerization observed was obviously not the result of epimerization at the carboxylate-bearing atom, which should have led to well-separated *trans* isomers.

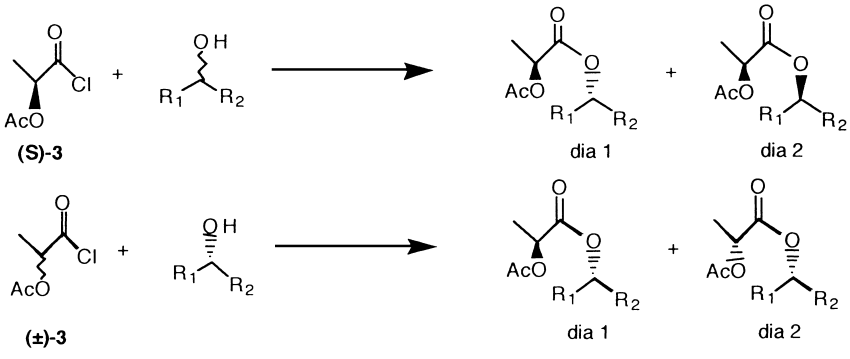


Scheme 2.

The effect of the base was further demonstrated by keeping a solution of (*S*)-*O*-acetyllactyl chloride and triethylamine in diethyl ether at room temperature for 10 h before addition of aniline at 4°C (Table 1, entry 6). An enantiomeric excess of 60% was observed for the amide derivative **5**, and the rate of epimerization depended on the amount of triethylamine. On the contrary, very little epimerization was observed using pyridine, even when the mixture was kept at 60°C for 2 h (Table 1, entry 7). The best conditions were to keep the temperature below 10°C during the addition of pyridine.

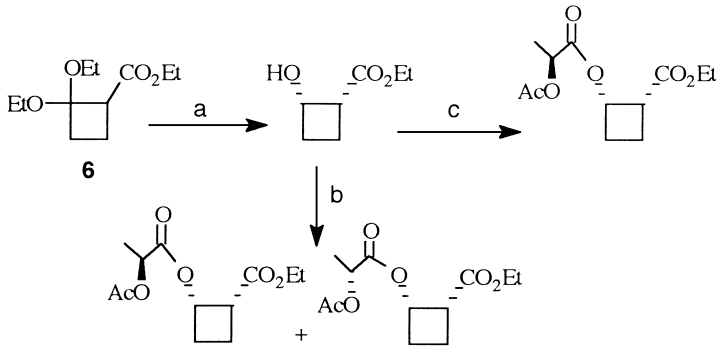
2.4. Use of the racemic O-acetyllactyl chloride

It is necessary to have at hand a mixture of diastereomeric acetyllactyl esters to determine the chromatographic conditions (GC: column, temperature or HPLC: column, solvant) for the best analytical separation. Generally, this mixture is obtained by derivatization of racemic alcohols with (*S*)-*O*-acetyllactyl chloride (Scheme 3, top). In some cases, for example, in asymmetric synthesis using microorganisms, the racemic alcohol samples are difficult or impossible to obtain. In such cases, the diastereomeric mixture can be easily obtained by reacting (±)-*O*-acetyllactyl chloride with the optically active alcohol (Scheme 3, bottom), preferably used in excess.



Scheme 3.

We have used this methodology in the ee measurement of hydroxyesters resulting from the baker's yeast reduction of the corresponding ketoester. An example is illustrated by the preparation of ethyl cyclobutanol 2-carboxylate,[26] which could only be obtained by incubation of the diethylacetal derivative **6** with baker's yeast, as the corresponding ketoester was highly unstable (Scheme 4).



Scheme 4. (a) Baker's yeast, H_2O, pH 2; (b) (±)-*O*-acetyllactyl chloride, 0.5 equiv.; (c) (*S*)-*O*-acetyllactyl chloride, 5 equiv.

The racemic *cis*- and *trans*-hydroxyesters were thus not accessible through chemical reduction. (±)-*O*-Acetyllactyl chloride derivatization of the microbiologically prepared *cis*-hydroxyester allowed to us find conditions for optimal GC separation and quantitative determination of diastereomeric derivatives.

3. Experimental

3.1. Instrumentation and chemicals

S-(+)-lactic acid (90%) from Fluka (reference no. 69773), containing about 30% condensation products and ≤1% *R*-(+)-lactic acid, was used throughout this work. All other chemicals and solvents were of the best quality available and used without purification. Chiral GC was performed on a Varian 3400 chromatograph equipped with a Chirasil-Val column (Altech, 50 m×0.32 mm; carrier gas He, 1 bar, 150°C).

3.2. Preparation of (S)-O-acetyllactyl chloride 3[6,7]

(*S*)-*O*-Acetyllactic acid **2**: 86 ml (95 g, 1.2 mol) of acetyl chloride was slowly added to 44 ml (52.8 g, 0.52 mol) of *S*-(+)-lactic acid **1** maintained in an ice-bath to keep the temperature below 10°C. After addition, the mixture was left overnight at room temperature, then evaporated in vacuo at 20°C, then at 50°C with an addition of toluene.

Determination of ee of (*S*)-*O*-acetyllactic acid: 0.1 ml of phenylisocyanate and 0.1 ml of dry pyridine were added to a solution of (*S*)-*O*-acetyllactic acid **2** (2 mg) in CH_2Cl_2 (1 ml). The mixture was left at room temperature for 4 h, and ethanol (0.1 ml) was added. After another 3 h, the precipitate was filtered and the filtrate analyzed by GC on the chiral column, (*R*): R_t=27.4 min, (*S*): R_t=28.5 min.

(*S*)-*O*-Acetyllactyl chloride **3**: 140 ml (228 g, 1.92 mol) of freshly distilled thionyl chloride was added dropwise to the resulting product **2** and the solution was left overnight at room temperature. Excess thionyl chloride was evaporated in vacuo at 40°C and the reaction product was distilled under vacuum at a bath temperature of 80°C to give pure (*S*)-*O*-acetyllactyl chloride (24 g, 31% yield). B.p. 38–40°C/8 mbar. $[\alpha]_D^{20}$ −34 (*c* 4, $CHCl_3$). ^{1}H NMR, δ (250 MHz, $CDCl_3$): 1.55 (3H, d, *J*=7.2 Hz, C*H*$_3$-CHO-),

2.11 (3H, s, C*H*$_3$-CO), 5.15 (1H, q, *J*=7.2 Hz, -C*H*O-). ^{13}C NMR, δ ($CDCl_3$): 16.14 (*C*H$_3$-CHO), 20.55 (*C*H$_3$-CO), 74.89 (CHO), 169.85 (CO), 172.75 (CO).

Determination of ee of (*S*)-*O*-acetyllactyl chloride: To 5 mg of **3** in solution in dry diethyl ether (1 ml) was added 0.1 ml of aniline at 4°C. After 1 h, 1 ml of H_2O was added. The organic layer was washed with 0.1N HCl, dried over Na_2SO_4 and analyzed by GC on the chiral column.

3.3. Derivatization procedure

Dry pyridine (0.1 ml) was added to a solution of about 5–8 mg of alcohol (or amino) compound in dry diethyl ether (1 ml) containing (*S*)-*O*-acetyllactyl chloride **3** (0.18 ml), at 4°C. The mixture was left for 10 min and then allowed to warm gradually to room temperature over 2 h. After washing twice with a saturated $NaHCO_3$ solution, then with 0.1N HCl, and then with a saturated NaCl solution, the organic phase was dried over Na_2SO_4, concentrated and directly analyzed by GC.

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