Pergamon

Tetrahedron: *Asymmetry* 10 (1999) 1729–99

TETRAHEDRON: *ASYMMETRY*

Application of the dimenthyl chlorophosphite for the chiral analysis of amines, amino acids and peptides

Oleg I. Kolodiazhnyi,* Oleg M. Demchuk and Alexander A. Gerschkovich

Institute of Bioorganic Chemistry, National Academy of Sciences of Ukraine, Murmanskaya Street, 1, Kiev 253094, Ukraine

Received 29 March 1999; accepted 12 April 1999

Abstract

The title reagent for the determination of the enantiomeric excess of chiral amino acids and peptides was prepared from (−)-(1*R*,2*S*,5*R*)-menthol and PCl_3. Its use as a chiral derivatizing agent for the determination of the enantiomeric content of amino acids and peptides by ^{31}P NMR is described.

1. Introduction

The determination of the enantiomeric purity of chiral amino acids and control of their racemization during peptide synthesis is an important process in the theoretical and practical study of asymmetric synthesis.[1,2] Different methods (polarimetry, chiral GC and HPLC, NMR in chiral medium, etc.) for *ee* determination of these compounds have been described.[3,4] Among these, the formation of diastereomers through the use of chiral derivatizing agents and determination of their ratio by NMR is a very useful and practical technique. Mosher's reagent, α-methoxy-α-(trifluoromethyl) phenylacetic acid, is currently the most widely employed in this case.[5]

There is a continuing need for the development of new chiral derivatizing agents for the determination of enantiomeric purities of amino acids and peptides potentially superior to Mosher's reagent. In this work we describe dimenthyl chlorophosphite as a convenient chiral derivatizing reagent. This reagent is convenient in that the phosphorus atom bearing two chiral menthyl groups is symmetric, therefore either retention or inversion at phosphorus during derivatization yields the same diastereomer (Scheme 1).

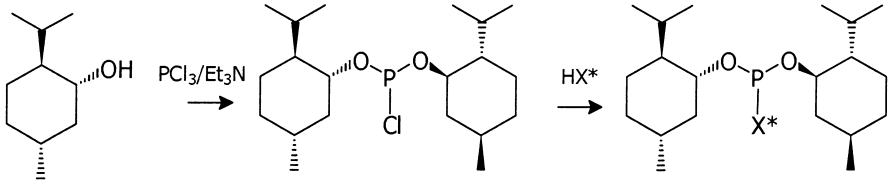
It is necessary to note that, although several chiral P(III) and P(V) chlorides have been examined previously as chiral derivatizing agents for alcohols, thiols, amines, and diols,[6] no method has been developed for the *ee* determination of amino acids and peptides.

The dimenthyl chlorophosphite can be prepared by reaction of PCl_3 with (−)-(1*R*,2*S*,5*R*)-menthol in ether at −20 → +20°C. This reagent is stable for weeks under an inert atmosphere and can be purified by

* Corresponding author. E-mail: oik@biophacs.kiev.ua

0957-4166/99/$ - see front matter
PII: S0957-4166(99)00139-1

distillation under vacuum. The preparation of dimenthyl chlorophosphite was accomplished according to the synthetic route outlined in Scheme 1.



HX= amine, amino acid ester or peptide

Scheme 1.

The dimenthyl chlorophosphite reacts easily with RNH_2 compounds. Very large differences in the ^{31}P NMR chemical shift ($\Delta\delta$) for the diastereomeric phosphonamides were observed allowing accurate integration and quantitative determination of the diastereomeric ratios. Analysis of the diastereomeric derivatives by ^{31}P NMR spectroscopy and the results obtained with different racemic and enantiomerically enriched amino acids or peptides are summarized in Table 1. An excellent agreement in all cases, with the *ee* measured using other analytical methods, has been found. The ^{31}P NMR spectroscopic observations reveal the effectiveness of the dimenthyl chlorophosphite for the determination of enantiomeric ratios in mixtures of racemic or enantiomerically enriched amino acids. The ^{31}P NMR spectra show well separated singlets corresponding to the two diastereomers (Fig. 1a). The enantiomeric purity can be accurately measured, and no kinetic resolution has been observed. Indeed, the diastereomeric pairs of derivatives were always observed in the case of racemic amino acids, and integration of the signals corresponded to the expected 50:50±2% ratios. A range of enantiomerically enriched mixtures of the (D)- and (L)-leucine enantiomers [(D):(L)=5:95, 20:80, 30:70, 40:60, 50:50] has been prepared. Integration of the NMR signals of these mixtures corresponded to the expected proportions with average deviation of ±2–5% (Fig. 1b).

One of the most useful characteristics of dimenthyl chlorophosphite is the substantial difference in ^{31}P NMR chemical shifts ($\Delta\delta$) exhibited by the resulting diastereomeric amidophosphites. The advantage of this reagent is the possibility to discriminate diastereomers even with a significant distance of the stereogenic center from the phosphorus atom (up to 10–11 atoms) (Table 1, compound **7a**). Mosher's acid in analogous conditions can discriminate an asymmetric center at a distance of 5–6 atoms from the CF_3 group.

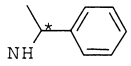
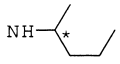
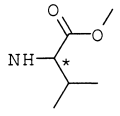
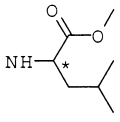
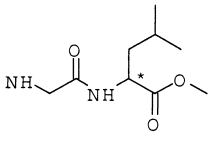
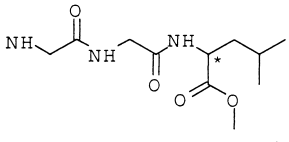
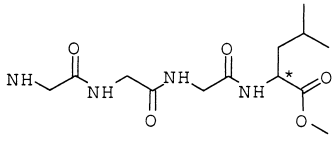
In view of the easy oxidation of trivalent phosphorus compounds in air, it is desirable to perform the spectroscopic studies of compounds directly after reaction completion. The oxidation of derivatized products by oxygen or elemental sulfur gives stable P(V) derivatives, which can also be used for the *ee* determination (Fig. 1c and Table 1, compounds **1b**, **7b**).

In conclusion, the new chiral derivatizing agent, dimenthyl chlorophosphite, is accessible, versatile and an effective derivatizing agent for the determination of the enantomeric excess of amines, amino acids and peptides bearing a free amino group.[7] It is easily prepared from cheap starting compounds and reacts readily with a wide range of amines, amino acids and simple peptides bearing free amino groups.

2. Experimental

Commercially available chiral (−)-(1*R*,2*S*,5*R*)-menthol of 98% enantiomeric purity was used in this work. Toluene was dried with phosphorus pentoxide and kept over sodium. Amino acid esters and peptides were prepared according to standard procedures[3b] or were obtained from commercial sources.

Table 1
Chiral analysis of some amines, amino acids and peptides

NN	X	δ_P of 1-7	$\Delta\delta_P$,ppm	Solvent
1a		142.30 *(R)* 143.10 *(S)*	0.8	Toluene
1b	----	68.46 *(R)* 68.59 (S)	0.13	Toluene
2a		143.84; 144.04	0.2	Toluene
3a		141.42 *(R)* 142.9 *(S)*	1.48	Toluene
4a		140.87*(R);* 142.24 *(S)*	1.37	Toluene
5a		139.87 *(R)* 140.55 *(S)*	0.68	$CDCl_3$
6a		137.57 *(R)* 138.46 *(S)*	0.89	$CDCl_3$
7a		137.77 *(R)* 137.87 *(S)*	0.10	$CDCl_3$
7b	----	69.44 *(R)* 69.57 *(S)*	0.13	Toluene

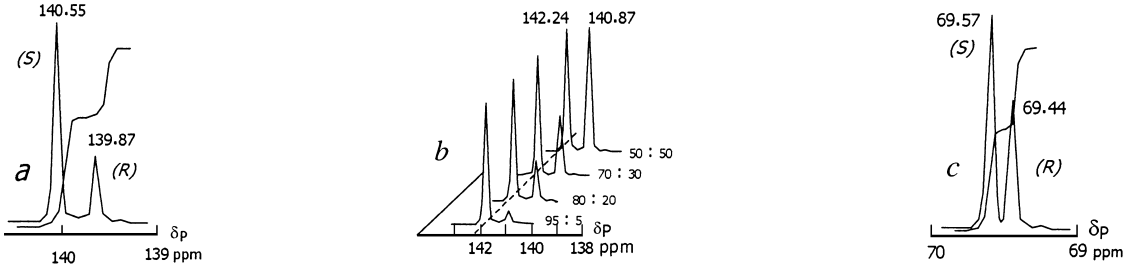


Figure 1. The ^{31}P NMR spectra of: (a) derivatized peptide **6a**; (b) enantiomerically enriched mixtures of the derivatized leucine ester **4a**; (c) derivatized peptide **7b**

The ^{31}P NMR spectra were recorded on a JEOL-90Q (40.5 MHz) and a Varian VXR-300 MHz (121 MHz) spectrometer using 85% H_3PO_4 as external standard.

2.1. Preparation of dimenthyl chlorophosphite

A solution of phosphorus trichloride (1.35 g, 0.01 mol) in dry diethyl ether (25 ml) was added dropwise with stirring under argon to a solution of (−)-(1*R*,2*S*,5*R*)-menthol (3.1 g, 0.2 mol) and 4 ml of triethylamine in 25 ml of diethyl ether at −20°C. The mixture was stirred for 2 h at room temperature. Then the triethylamine chlorohydrate was filtered off and the solvent was evaporated under reduced pressure. The residue was purified by distillation under vacuum. Yield of 80%, bp 125°C (0.02 mmHg); $[\alpha]_D$ −83.8 (3.6, toluene); ^{31}P NMR (δ, ppm, $CDCl_3$): 145 (t, $^3J_{PH}$ 10 Hz); found (%): Cl 7.81; $C_{20}H_{38}ClO_2P$ calculated (%): Cl 7.62.

2.2. Procedure for the determination of the enantiomeric purity of chiral RNH_2 compounds with dimenthyl chlorophosphite to give diastereomeric amidophosphites

A solution (5 ml) of dimenthylchlorophosphite (0.001 mol) in the appropriate solvent was introduced into a round bottomed flask and a solution of Et_3N (0.002 mol) and amino acid ester or peptide (0.001 mol) was added slowly. After the addition the mixture was stirred at 0°C. When the formation of the derivative was complete (~5 min), the suspension was filtered, transferred into an NMR tube along with 0.1 ml of C_6D_6 for locking. After that the ^{31}P NMR spectrum was recorded.

References

1. (a) Kolodiazhnyi, O. I. *Tetrahedron: Asymmetry* **1998**, *9*, 1279; (b) Kolodiazhnyi, O. I. New Achievement in Asymmetric Synthesis of Organophosphorus Compounds. In *Advances in Asymmetric Synthesis*; Hassner, A., Ed.; JAI: Greenwich, CT, 1998; Vol. 3, pp. 273–357.
2. Valentine Jr., D. *Asymmetric Synthesis*; Morrison, J. D.; Scott, J. W., Eds.; Academic Press: New York, 1984; Vol. 4, pp. 267–312.
3. (a) Kamp, D. S. Racemization in Peptide Synthesis. In *The Peptides. Analysis, Synthesis, Biology*; Gross, E.; Meienhofer, J., Eds.; Academic Press, Harcourty Brace Jovanovich, Publishers: Orlando, San Diego, New York, 1979; Vol. 1, pp. 315–383. (b) Gerschkowich, A. A., Kibirev, V. K. *Chemical Synthesis of Peptides*; Naukova Dumka Publ.: Kiev, 1992; p. 360.
4. Takeuchi, Y.; Itoh, N.; Satoh, T.; Koizumi, T.; Yamaguchi, K. *J. Org. Chem*. **1993**, *58*, 1812–1820.
5. Dale, J. A.; Dull, D. L.; Mosher, H. S. *J. Org. Chem*. **1969**, *34*, 2543.
6. (a) Brunel, J. M.; Faure, B. *Tetrahedron: Asymmetry* **1995**, *6*, 2353–2356; (b) Brunel, J. M.; Pardigon, O.; Maffel, M.; Buono, G. *Tetrahedron: Asymmetry* **1992**, *3*, 1243–1246; (c) Alexakis, A.; Mutti, S.; Normand, J. F.; Mangeney, P. *Tetrahedron: Asymmetry* **1990**, *1*, 437–440; (d) Alexakis, A.; Mutti, S.; Mangeney, P. *J. Org. Chem*. **1994**, *59*, 3326–3334.
7. We also used dimenthyl chlorophosphite for the chiral analysis of alcohols and thiols. See: Kolodiazhnyi, O. I.; Demchuk, O. Submitted for publication. The determinations of *ee* of some alcohols are shown in the table:

Alcohol	δ_P	$\Delta\delta_P$	Solvent
Et(Me)CHOH	142.23; 142.50	0.27	Toluene
$Et_2NCH(Me)CH_2OH$	140.54; 140.88	0.34	Toluene
$(EtO)_2P(O)CH(Ph)OH$	142.83; 143.37	0.46	Toluene
Menthol	143.17; 143.44	0.27	Toluene