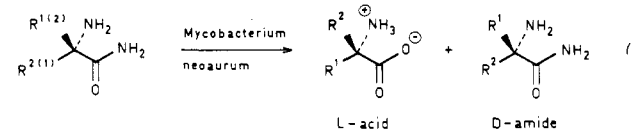
Communications

Synthesis of Optically Pure α-Alkylated α-Amino Acids and a Single-Step Method for Enantiomeric Excess Determination

Summary: A method for the enzymatic resolution of the amides of some racemic α-alkylated amino acids is described as well as a method involving derivatization with (*S*)-2-chloropropionyl chloride followed by ^{1}H NMR analysis to establish the enantiomeric excesses of the free amino acids.

Sir: New optically pure amino acids are available through a combination of organic synthesis for preparation of racemic material followed by the use of a broad-specificity peptidase to achieve resolution on a large scale.[1] In conjunction with this work, simple, fast, and reliable methods for determinations of the enantiomeric excesses (ee) are required. Optically pure α-alkylated α-amino acids are of special interest as α-H-amino acid antagonists.[2] In addition, the conformational freedom of the derived peptides is restricted and the tendency toward α-helix formation may be promoted.[3] Several routes to optically pure α-alkylated α-amino acids have been elaborated;[4] we describe here an economically attractive and broadly applicable synthesis suitable for large-scale production as well as a simple procedure based on derivative formation and ^{1}H NMR spectroscopy for determination of the ee's.

The syntheses of racemic α-alkylated α-amino acid *amides* are carried out by known literature procedures.[5] On treatment with freeze-dried cells obtained from commercially available *Mycobacterium neoaurum* ATCC 25795, stereoselective hydrolysis occurs (eq 1).[5] Separation is achieved by extraction or by the use of ion-exchange columns. Some of the L-acids prepared, together with the rotations, are shown. These rotations are those of the enantiomerically pure L-acids (see further for establishment of the ee's). The corresponding D-acids have also been prepared.



(1)

R^1	R^2	rotations, L-acids
$(CH_3)_2CH$	CH_3	−4.0° (*c* 1.3, H_2O)
$(CH_3)_2CHCH_2$	CH_3	+34.2° (*c* 3, H_2O)
C_6H_5	CH_3	−86° (*c* 1, 1 N HCl)
$C_6H_5CH_2$	CH_3	−22.0° (*c* 1, H_2O)
$4\text{-}CH_3OC_6H_4CH_2$	CH_3	−6.9° (*c* 1, HCl)
$C_6H_5(CH_2)_2$	CH_3	+38.1° (*c* 1, HCO_2H)
$C_6H_5CH_2$	C_2H_5	−22.8° (*c* 2, H_2O)

The resolution of α-methylleucine is described as an example. Racemic α-methylleucine amide (144 g, 1 mol) is dissolved in 1.2 L of H_2O, and the preparation of whole cells (30.0 g) containing aminopeptidase (about 10% of the weight) is added. After incubation for 72 h at 37 °C, the biocatalyst is removed by centrifugation. The white solid (142 g) that remains after evaporation of solvent is stirred with 600 mL of $CHCl_3$. The suspension is filtered and washed three times with 50-mL portions of $CHCl_3$. On evaporation of the $CHCl_3$ extract, 70.3 g (0.488 mol, 97.6% yield) of the unreacted amide of D-α-methylleucine is obtained, $[\alpha]^{20}_D$ −16.9° (*c* 1, H_2O). Hydrolysis (6 N HCl, 72 h, 90 °C) gave D-α-methylleucine in 97.3% yield, $[\alpha]^{20}_D$ −34.2° (*c* 3, H_2O). The L-amino acid (71.8 g, 0.5 mol, 99% yield) has $[\alpha]^{20}_D$ +32.8° (*c* 3, H_2O) and is insoluble in $CHCl_3$. It is slightly contaminated with the amide. After passage over an ion-exchange column (CH_3CO_2H followed by H_2O), pure L-α-methylleucine, $[\alpha]^{20}_D$ +34.2° (*c* 3, H_2O), is obtained.[6]

A procedure for ee determination was described recently wherein *esters* of amino acids are coupled with the achiral reagent CH_3PSCl_2.[7] Analysis of the ^{31}P NMR spectrum of the mixture of diastereomers obtained allows determination of the ee's. The extra steric hindrance present in α-alkylated α-amino acids unfortunately inhibits both the esterification and the coupling with CH_3PSCl_2.[8] We find, however, that Schotten–Baumann coupling of (*S*)-2-chloropropionyl chloride, available from (*S*)-2-chloropropionic acid obtained from diazotization of (*S*)-alanine,[9]

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(2) (a) Barrett, G. C., *Amino-Acids, Pept., Proteins* **1979**, *10*, 11. (b) Schollkopf, U. *Tetrahedron* **1983**, *39*, 2085. See also: (c) Reinhold, D. F.; Firestone, R. A.; Gaines, W. A.; Chemerda, J. M.; Sletzinger, M. *J. Org. Chem.* **1968**, *33*, 1209 for L-methyl-DOPA. (d) Eur. Patent 0.123.830, 1984, and Eur. Patent 0.169.300, 1985, to Cyanamide Corp. for D-α-methylvaline amide. (e) Pandey, R. C.; Meng, H.; Cook, J. C.; Rinehart, K. L. *J. Am. Chem. Soc.* **1977**, *99*, 5203 for L-isovaline. (f) Bollinger, F. W. *J. Med. Chem.* **1971**, *14*, 373 for L-α-methyltyrosine.

(3) Weihrauch, T.; Leibfritz, D. *Liebigs Ann. Chem.* **1985**, 1917.

(4) (a) Schollkopf, U. *Top. Current Chem.* **1983**, *109*, 65. (b) Schollkopf, U.; Tolle, R.; Egert, E.; Nieger, M. *Liebigs Ann. Chem.* **1987**, 399. (c) Seebach, D.; Imwinkelried, R.; Weber, T. In *Modern Synthetic Methods*; Springer-Verlag: Berlin, 1986; pp 125–129. (d) Bajgrowicz, J. A.; Lossec, B.; Pigiere, Ch.; Jacquier, R.; Viallefont, Ph. *Tetrahedron Lett.* **1983**, *24*, 3721. (e) Kolb, M.; Barth, J. *Tetrahedron Lett.* **1979**, 2999. (f) Yamada, S.; Oguri, T.; Shiori, T. *J. Chem. Soc., Chem. Commun.* **1976**, 136. (g) Pey, P.; Vevert, J. P.; van Dorsslaer, V.; Kolb, M. *J. Org. Chem.* **1979**, *44*, 2732. (h) Seebach, D.; Aebi, J. D.; Naef, R.; Weber, T. *Helv. Chim. Acta* **1985**, *68*, 144. (i) Fadel, A.; Salaun, J. *Tetrahedron Lett.* **1987**, *28*, 2243. (j) Karady, S.; Amato, J. S.; Weinstock, J. M. *Tetrahedron Lett.* **1984**, *25*, 4337. (k) Bajgrowicz, J. A.; Cossec, B.; Pigiere, Ch.; Jacquier, R.; Viallefont, Ph. *Tetrahedron Lett.* **1984**, *25*, 1789.

(5) Boesten, W. H. J.; Peters, P. J. H. *Eur. Patent* 1.508.54, 1984; *Chem. Abstr.* **1986**, *104*, 128238s. We purchased the enzyme from Novo Chemicals.

(6) Full experimental details are given in the microfilm edition of this journal.

(7) Feringa, B. L.; Strijtveen, B.; Kellogg, R. M. *J. Org. Chem* **1986**, *51*, 5484.

(8) (a) Wipf, P.; Heimgartner, H. *Helv. Chim. Acta* **1987**, *70*, 354. (b) Both the esterification of *α-alkylated* amino acids with thionyl chloride/methanol as well as the condensation of the amides with benzaldehyde (a step in the separation of enantiomers using a peptidase[1b]) are much slower than the analogous reactions with the corresponding α-H substituted derivatives.

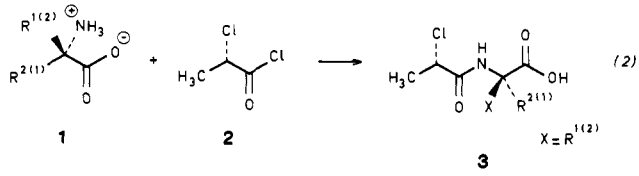
(9) (a) Freudenberg, K.; Markert, L. *Chem. Ber.* **1927**, *60*, 2447. (b) Fu, S. C.; Birnbaum, S. M.; Greenstein, P. J. *J. Am. Chem. Soc.* **1954**, *76*, 6054. (c) The enantiomeric purity of (*S*)-2-chloropropionic acid obtained by this synthetic route is known to be very high: Wataba, K.; Chang, S.-C.; Gil-Av, E.; Koppenhoefer, B. *Synthesis* **1987**, 225.

0022-3263/88/1953-1826$01.50/0

Table I. ^{1}H NMR Data for (*S*)-2-Chloropropionyl Derivatives of Racemic Amines and Amino Acids

entry	amino acids	chemical shift, δ CH_3CHCl	chemical shift, δ $NHCCH_3$	solvent
1	$(CH_3)_2CHCHNH_2CO_2H$	1.79, 1.77 (J = 7.0 Hz)		CD_3OD
		1.75, 1.74 (J = 7.0 Hz)		$CDCl_3$
		1.51, 1.49 (J = 6.8 Hz)		C_6D_6
2	$C_6H_5CH_2CHNH_2CO_2H$	1.73, 1.67 (J = 6.7 Hz)		CD_3OD
		1.68, 1.63 (J = 7.0 Hz)		$CDCl_3$
3	$CH_3SCH_2CH_2CHNH_2CO_2H$	no separation		$CDCl_3$
		1.60, 1.57 (J = 7.0 Hz)		C_6D_6
		1.79, 1.78 (J = 7.0 Hz)		CD_3OD
4	$C_6H_5C(CH_3)NH_2CO_2H$	1.76, 1.72 (J = 7.4 Hz)		$CDCl_3$
5	$C_6H_5C(CH_3)NH_2CO_2H$	1.73, 1.69 (J = 7.3 Hz)		$CDCl_3$
6	$(CH_3)_2CHC(CH_3)NH_2CO_2H$	1.76, 1.74 (J = 7.0 Hz)	1.64, 1.62	CD_3OD
7	$C_6H_5CH_2C(CH_3)NH_2CO_2H$	no separation	1.69, 1.60	CD_3OD
		no separation	1.62, 1.59	D_2O
8	$C_6H_5CH_2CH_2C(CH_3)NH_2CO_2H$	no separation	1.74, 1.71	CD_3OD
		no separation	1.58, 1.54	C_6D_6
9	p-$CH_3OC_6H_4CH_2C(CH_3)NH_2CO_2H$	no separation	1.68, 1.60	CD_3OD
10	$(CH_3)_2CHCH_2C(CH_3)NH_2CO_2H$	no separation	1.67, 1.66	CD_3OD
			1.78, 1.76	C_6D_6

with the free amino acids followed by analysis of the ratio of diastereomers by ^{1}H NMR provides a fast and accurate analysis of the ee's of normal as well as α-alkylated amino acids (eq 2).[10]



(2)

The ee determination of α-methylphenylglycine serves as an example. A mixture consisting of 26% *R* and 74% *S* was prepared by weighing the appropriate amounts. This mixture was treated with freshly prepared (*S*)-2-chloropropionyl chloride.[11] The 200-MHz ^{1}H NMR spectrum of this N-acylated product of **2** in $CDCl_3$ revealed two doublets for the CH_3CHCl absorptions at δ 1.69 and 1.73, both J = 7.2 Hz. Integration gave a value of 25 ± 2% minor diastereomer and 75 ± 2% major diastereomer. No resolvable separations of absorptions of the diastereomers formed from reaction of the amino acid with (*S*)-2-bromopropionyl chloride or (*R*)-2-methoxy-2-phenyl-3,3,3-trifluoropropionyl chloride (Mosher's reagent) were observed. This reagent may be used, however, under Schotten–Baumann conditions with common amino acids.[10b]

Pertinent data for various *d,l*-amino acids are collected in Table I. For all cases reported in the table, mixtures varying in molar composition between 60/40 and 70/30 were made from optically pure enantiomers by weighing and the ee's of the mixtures (unknown beforehand to the operators) were determined by the ^{1}H NMR method. Agreement was always within ±2%.

The α-alkylated amino acids are particularly challenging. Obviously, however, by proper choice of solvent and signal (see entries 6 and 10), sufficient signal separation can be obtained to allow accurate integration of the diastereomer ratios. These diastereomer ratios reflect accurately the enantiomeric ratios, indicative of the fact that no measurable amount of kinetic resolution occurs during acylation of the acids. We believe that the methodology described here provides a simple entry to the large-scale syntheses of optically pure α-alkylated α-amino acids and an easy, accurate method for ascertaining the enantiomeric excesses of α-amino acids in general.

Supplementary Material Available: Description of experimental details (2 pages). Ordering information is given on any current masthead page.

(10) For some recent methods involving acylation procedures for determination of enantiomeric (or diastereomeric for peptides) excesses of amino acids and amines, see the following. (a) Chiral amines: Nagao, Y.; Yagi, M.; Ikeda, T.; Fujita, E. *Tetrahedron Lett.* **1982**, *23*, 205. (b) By use of Mosher's acid and ^{19}F NMR spectroscopy for ee determination of amino acids and extent of racemization for small peptides: Breuer, W.; Ugi, I. *J. Chem. Res., Synop.* **1982**, 271. Hull, W. E.; Seeholzer, K.; Baumeister, M.; Ugi, I.; *Tetrahedron* **1986**, *42*, 547. (c) Use of dibenzoyl-L-tartaric anhydride for ee determinations of chiral amines, hydroxylamines, amino acid *esters*, and peptides (as C-terminal *esters*): Kolasa, T.; Miller, M. J. *J. Org. Chem.* **1986**, *51*, 3055. (d) Observation of differing methyl absorptions from alanyl residues in ^{1}H NMR as a means of detecting racemization: Halpern, B.; Nitecki, D. E.; Weinstein, B. *Tetrahedron Lett.* **1967**, 3075. Weinstein, B.; Dritchard, A. E. *J. Chem. Soc., Perkin Trans. 1* **1972**, 1015. Halpern, B.; Westley, J. W.; Weinstein, B. *J. Chem. Soc., Chem. Commun.* **1967**, 160. (e) Differing absorptions of *methyl esters* of C-terminal ends of peptides for detection of racemization: Benoiton, N. L.; Kuroda, K.; Chen, F. M. F. *Int. J. Pept. Protein Res.* **1979**, *13*, 403. Nagaraj, R.; Balaram, P. *Tetrahedron* **1981**, *37*, 2001. Davies, J. S.; Thomas, R. J.; Williams, M. K. *J. Chem. Soc., Chem. Commun.* **1975**, 76.

(11) The amino acid (0.5 mmol) is dissolved in 1 mL of 2.5 N KOH solution. The solution is cooled to −10 °C, and **2** (0.75 mmol) is added dropwise with a syringe. The solution is stirred for 10 min and is brought to neutral pH with 2 N HCl. The solution is extracted with ethyl acetate, and the organic layer is dried and then evaporated. The amide is then taken up in the solvent of choice for NMR determination.

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