(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
9 February 2017 (09.02.2017)

WIPO | PCT

(10) International Publication Number
WO 2017/021378 A1

(51) International Patent Classification:
C07D 207/24 (2006.01) *C07D 207/16* (2006.01)

(21) International Application Number:
PCT/EP2016/068347

(22) International Filing Date:
1 August 2016 (01.08.2016)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
102015000041444 3 August 2015 (03.08.2015) IT

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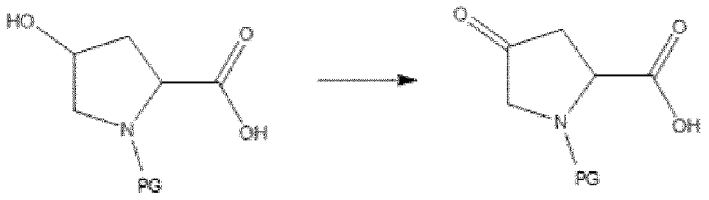
(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, ST, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

— *with international search report (Art. 21(3))*

(54) **Title**: PROCESS FOR THE SYNTHESIS OF PROTECTED 4-KETO-PROLINE



protected 4-keto-proline
(1)

(57) **Abstract**: Disclosed is a process for the synthesis of N-protected 4-keto-prolines (1), starting with the corresponding protected 4-hydroxyproline, in the presence of the oxidising system P_2O_5/dimethylsulphoxide (DMSO). Said process can also be applied in the presence of a chiral centre in the position bearing carboxylic acid (the 3 position) without significant epimerisation of said centre. Said process is particularly advantageous from the environmental standpoint and in terms of yields, productivity and the purity of the product obtained, since it does not use chromium-based oxidants, metals such as ruthenium, or oxidising systems which are precursors of genotoxic impurities.

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PROCESS FOR THE SYNTHESIS OF PROTECTED 4-KETO-PROLINE

The invention relates to a process for the preparation of N-protected 4-keto-prolines by oxidation of the corresponding N-protected 4-hydroxyprolines.

BACKGROUND TO THE INVENTION

4-Keto-proline derivatives are important intermediates for the preparation of (*inter alia*) ACE inhibitors, which are used to treat hypertension and cardiovascular disease.

As reported in the literature (JACS 1957, 79, 185-92), 4-keto-proline derivatives are unstable, especially under basic conditions, where they can give rise to aldol condensation or ring opening, generating by-products and impurities.

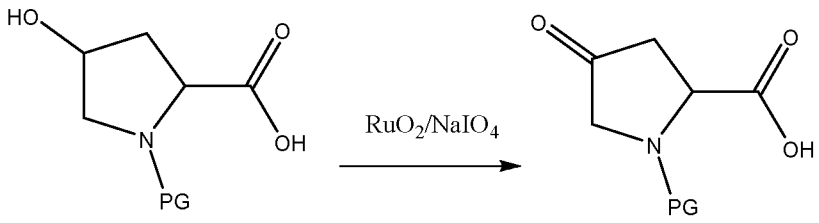
The synthesis of 4-keto-proline intermediates by oxidation from the corresponding hydroxyl derivatives is described in various patents and articles.

DD283626A5 discloses the oxidation of N-benzyloxy-4-hydroxyproline via a pyridine-SO_3 complex. Although the oxidation described takes place in the absence of metals, its main drawback is the use of pyridine, which is highly toxic. The same reagent is used in the presence of an organic base in CN 102 453 033 to oxidise an N-protected 4-hydroxyproline.

Conventional oxidation methods are also reported which use the chromium-based Jones reagent, the system most widely used for oxidation of N-protected 4-hydroxyprolines. Said oxidative systems containing chromium are highly toxic (*US4296113, JOC2001, 66, 3593; JOC2002,67,7162*).

Organic Process Research & Development, 19(1), 270-283; ***2015*** describes oxidation of N-BOC hydroxyproline using TEMPO/hypochlorite as oxidising system, which can generate genotoxic impurities.

US 8618310 discloses oxidation in homogenous aqueous phase in the presence of RuO_2 and $NaIO_4$ according to the following scheme:



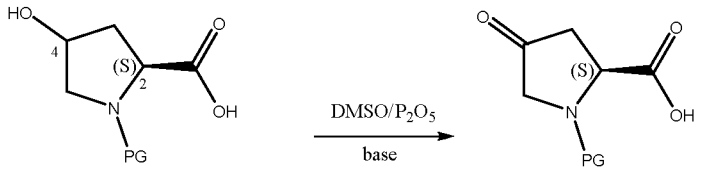
The product formed precipitates, thus preventing further oxidation and degradation reactions. Although from the toxicological and environmental standpoint said oxidation has a lower impact than the processes described in the literature cited above, it uses ruthenium which, as well as having a certain economic impact, also requires strict monitoring in the isolated product to limit its contamination by heavy metals.

The oxidising system P_2O_5/DMSO has been used on substrates other than N-protected 4-hydroxyprolines, in the presence or absence of a base (Synthesis, Georg Thieme verlag, Stuttgart, no. 10, 1-10-1990, 857-870; Claudio Palomo et al.: "New Synthesis of a-Amino Acid N- Carboxy Anhydrides through Baeyer-Villiger Oxidation of a-Keto & Lactamst", J. Org. Chem. Ber. Dtsch. Chem. Ges.. Dtsch. Chem. Ges. J. P.;Winitz, M. Chemistry of the Amino Acids, 15-4-1994, 39-861).

DESCRIPTION OF THE INVENTION

An advantageous oxidation process for 4-hydroxyproline derivatives has now been found wherein oxidation is obtained with the P_2O_5/DMSO oxidising system under basic conditions, with subsequent isolation under controlled conditions to prevent the degradation thereof known from the literature.

The process according to the invention is shown in the scheme below:



wherein PG is an amino-protecting group (as described in the book "Protective Groups in Organic Synthesis, Third Edition. T.W. Greene") which comprises a carbonyl function and is bonded via said function to the amino nitrogen.

One of the most common groups is tert-butyloxycarbonyl (BOC).

The process consists of a single oxidation stage followed by isolation of the product. The product can be purified as salt with organic or inorganic bases and then isolated again as carboxylic acid.

Oxidation takes place in a mixture of organic solvents, such as a mixture of dichloromethane and dimethylsulphoxide, or in pure dimethylsulphoxide.

Oxidation takes place by consecutive additions of phosphoric anhydride (P_2O_5) and an organic base such as diisopropylethylamine or triethylamine.

When the conversion has been completed, the reaction mixture is quenched and extracted in organic solvent.

The reaction product is then precipitated from the end-of-workup concentrated organic solution in salified form, for example as phenylethylamine, naphthylamine or dicyclohexylamine salt.

Subsequent desalification in an organic solvent such as methyl tert-butyl ether and subsequent precipitation from acetonitrile provides the desired product of oxidation with high purity.

According to a preferred embodiment of the invention, the process is performed as follows:

1 mole of N-protected hydroxyproline is basified with 1.0-1.1 moles of organic base, preferably diisopropylamine in dimethylsulphoxide. 5-25 volumes of solvent are used, preferably 14-16 volumes relative to the quantity of N-protected hydroxyproline, or mixtures of dimethylsulphoxide/methylene chloride. At a temperature of under 25°C, preferably at a temperature ranging between 15°C and 18°C, the organic base, preferably diisopropylamine, is added in portions, in aliquots of 0.25-0.65 moles at a time, preferably 0.4-0.5 moles, for a total of 7-18 portions, preferably 9-11 portions, followed, in rapid succession, by portions of phosphoric anhydride, in aliquots of 0.08-0.21 moles at a time, preferably 0.14-0.17 moles, for a total of 7-18 portions, preferably 9-11 portions, at a temperature of under 25°C,

preferably at a temperature ranging between 15°C and 20°C.

The reaction is monitored by UPLC analysis, using an ACQUITY BEH C18 column and water/acetonitrile/0.1% trifluoroacetic acid as eluent phase.

When the reaction is completed, the mixture is acidified to a pH ranging between 2.0-4.0, preferably 2.5-3.5, with hydrochloric acid.

The mixture is then extracted with an organic solvent, preferably methylene chloride, and the aqueous phase is back-extracted to recover the product with the same solvent. The oil obtained after concentration can be used directly in the subsequent salification stage.

The oil obtained is dissolved in organic solvent, preferably ethyl acetate. 5-8 volumes of solvent are used, preferably 6-7 volumes relative to the initial quantity of N-protected hydroxyproline. 1.5-2.5 moles of organic base, phenylethylamine or naphthylamine or dicyclohexylamine, are added to the resulting solution, at a temperature of under 25°C, preferably between 20°C and 25°C. Said moles are calculated on the N-protected hydroxyproline content present in the titrated starting oil.

The resulting suspension is filtered. The solid thus obtained can be further purified if necessary by recrystallisation or triturating with ethyl acetate or other known solvents.

The N-protected 4-keto-proline acid salt is then dissolved in 3-5 volumes of water and 4-6 volumes of organic solvent, preferably methyl tert-butyl ether. The resulting suspension is acidified with inorganic acids, preferably phosphoric acid, and the organic phase is evaporated until dry. The oil obtained is dissolved in 3-5 volumes relative to the starting salt in organic solvent, preferably ethyl acetate.

The filtered solid is dried under vacuum at the temperature of 45°C-55°C to obtain N-protected 4-keto-proline.

The solid thus obtained can be further purified if necessary by recrystallisation or trituration from ethyl acetate or other organic solvents known

from the literature.

The process according to the invention is particularly advantageous as, unlike known processes, it is carried out without the use of oxidising systems containing harmful or toxic substances, and in the absence of metals whose use must be strictly monitored in the isolated product. Moreover, from the economic standpoint said oxidation presents the advantage of using reagents with a low cost impact, the by-products of which are easily removed in aqueous phase.

Finally, said process is safe in calorimetric terms, and is therefore industrially scalable.

The invention is illustrated in detail in the following examples.

Example 1: synthesis of N-BOC-4-keto-proline naphthylamine salt

Diisopropylamine (14.0 g, 0.108 moles) is added to a solution of N-BOC hydroxyproline (25 g, 0.108 moles) in dimethylsulphoxide (350 ml). The resulting solution is cooled to 16-17°C, and aliquots of diisopropylamine (6.28 g, 0.0486 moles) are added in about 10 portions, followed by corresponding aliquots of phosphoric anhydride (4.60 g, 0.0162 moles).

The reaction is then monitored by UPLC. When the reaction has ended, the reaction mixture is slowly dripped into a mixture of methylene chloride (250 ml) and water (30 ml) at pH 3.0±0.5, at a temperature of 0-5°C, maintaining the pH constant between 3.0±0.5 by simultaneous addition of hydrochloric acid (about 40 ml).

When the phases have been separated the aqueous phase is back-extracted with dichloromethane (150 ml), and the combined organic phases are washed with water (2x100 ml).

The mixture is then concentrated to an oil by drying with ethyl acetate.

The resulting oil is dissolved in ethyl acetate (162 ml). Naphthylamine (18.6 g, 0.13 moles) is added to the resulting solution at a temperature of 20-25°C. The resulting product is filtered and washed with ethyl acetate (41 ml).

The isolated solid is dried at low pressure at 50°C to obtain N-BOC-4-keto-proline naphthylamine salt (24.2 g, 0.065 mol) as a white solid. Molar yield from N-BOC-hydroxyproline: 60%.

Example 2: synthesis of N-BOC-4-keto-proline from N-BOC-4-keto-proline naphthylamine salt

23.2 g of N-protected 4-keto-proline naphthylamine salt (93%, 0.058 mol) is suspended in a mixture of 100 ml of water and 250 ml of methyl tert-butyl ether. 85% phosphoric acid is added to the suspension until a pH of 2±0.5 is reached. The phases are separated and the aqueous phase is back-extracted with methyl tert-butyl ether (125 ml). The combined organic phases are then washed with water (50 ml) and evaporated until dry, drying with ethyl acetate (3x50 ml). Ethyl acetate (25 ml) is added to the resulting oily residue, and the mixture is stirred at 25±5°C for 1 h. The solid is then filtered, and washed with ethyl acetate (10 ml). The resulting product is then dried at low pressure at the temperature of 50°C to obtain N-protected 4-keto-proline (12.2 g, 0.053 mol) as a white solid. HPLC purity: 99.5%

Molar yield from N-BOC-keto-proline naphthylamine salt: 91%.

The solid thus obtained can be further purified if necessary by recrystallisation or trituration from ethyl acetate or other known organic solvents.

UPLC-MS [M+H+] = 228

1H NMR (400 MHz, DMSO)

δ 12.85 (s, 1H), 4.54 (m, 1H), 3.86-3.78 (m, 1H), 3.69-3.64 (m, 1H), 3.16-3.06 (m, 1H), 2.53-2.45 (m, 1H + DMSO), 1.42 and 1.40 (2 × s, 9H).

Example 3: synthesis of N-BOC-4-keto-proline dicyclohexylamine salt

Diisopropylamine (21.0 g, 0.162 moles) is added to a solution of N-BOC hydroxyproline (37.5 g, 0.162 moles) in dimethylsulphoxide (530 ml). The resulting solution is then cooled to 16-17°C and aliquots of diisopropylamine (9.42 g, 0.0729 moles) are added in about 10 portions, followed by corresponding aliquots of phosphoric anhydride (6.90 g, 0.0243 moles).

The reaction is then monitored by UPLC. When the reaction has ended, the reaction mixture is slowly dripped into a mixture of methylene chloride (400 ml) and water (45 ml) at pH 3.0±0.5, at a temperature of 0-5°C, maintaining the pH constant between 3.0±0.5 by simultaneous addition of hydrochloric acid (about 60 ml).

When the phases have been separated the aqueous phase is back-extracted with dichloromethane (230 ml), and the combined organic phases are washed with water (2x150 ml).

The mixture is then concentrated to an oil by drying with ethyl acetate.

The resulting oil is dissolved in ethyl acetate (250 ml). Dicyclohexylamine (35.3 g, 0.2 moles) is added to the resulting solution at a temperature of 20-25°C. The resulting product is filtered, and washed with ethyl acetate (60 ml).

The isolated solid is dried at low pressure at 50°C to obtain N-BOC-4-keto-proline dicyclohexylamine salt (45.2 g, 0.11 mol) as a white solid.

Molar yield from N-BOC-hydroxyproline: 68%.

Example 4: synthesis of N-BOC-4-keto-proline from N-BOC-4-keto-proline dicyclohexylamine salt

40 g of N-protected 4-keto-proline dicyclohexylamine salt (93%, 0.09 mol) is suspended in a mixture of 155 ml of water and 390 ml of methyl tert-butyl ether. 85% phosphoric acid is added to the suspension until a pH of 2±0.5 is reached. The phases are separated and the aqueous phase is back-extracted with methyl tert-butyl ether (200 ml). The combined organic phases are then washed with water (80 ml) and evaporated until dry, drying with ethyl acetate (3x80 ml). Ethyl acetate (31 ml) is added to the resulting oily residue, and the mixture is stirred at 25±5°C for 1 h. The solid is then filtered, and washed with ethyl acetate (15 ml). The resulting product is then dried at low pressure at the temperature of 50°C to obtain N-protected 4-keto-proline (19 g, 0.083 mol) as a white solid. HPLC purity: 99.6%.

Molar yield from N-BOC-keto-proline dicyclohexylamine salt: 92%.

The solid thus obtained can be further purified if necessary by recrystallisation or trituration from ethyl acetate or other known organic solvents.

UPLC-MS [M+H+] = 228

1H NMR (400 MHz, DMSO)

δ 12.85 (s, 1H), 4.54 (m, 1H), 3.86-3.78 (m, 1H), 3.69-3.64 (m, 1H), 3.16-3.06 (m, 1H), 2.53-2.45 (m, 1H + DMSO), 1.42 and 1.40 (2 × s, 9H).

CLAIMS

1. A process for the preparation of N-protected 4-keto-prolines comprising the oxidation of the corresponding N-protected 4-hydroxy-prolines by means of P_2O_5/dimethylsulphoxide (DMSO) in basic conditions.

2. A process according to claim 1 wherein the oxidation is carried out in a mixture of dichloromethane and dimethylsulphoxide or in pure dimethylsulphoxide.

3. A process according to claim 2 wherein the oxidation is carried out by means of consecutive additions of phosphoric anhydride ($P_2O_{5)}$ and an organic base.

4. A process according to claim 3 wherein the organic base is diisopropylethylamine or triethylamine.

5. A process according to one or more of claims 1 to 4 wherein the N-protected 4-keto-proline is N-BOC 4-keto-proline and the N-protected 4-hydroxy-proline is N-BOC 4-hydroxy-proline.

6. A process according to one or more of claims 1 to 5 wherein the product is purified as salt with organic or inorganic bases and then isolated again as carboxylic acid.

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2016/068347

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07D207/24 C07D207/16
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07D

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
EPO-Internal, CHEM ABS Data, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	CN 102 453 033 A (SHANGHAI INST PHARM INDUSTRY) 16 May 2012 (2012-05-16) paragraph [0043] - paragraph [0045]	1-6
Y	TIDWELL T T: "OXIDATION OF ALCOHOLS BY ACTIVATED DIMETHYL SULFOXIDE AND RELATED REACTIONS: AN UPDATE", SYNTHESIS, GEORG THIEME VERLAG, STUTTGART, DE, no. 10, 1 October 1990 (1990-10-01), pages 857-870, XP000160078, ISSN: 0039-7881, DOI: 10.1055/S-1990-27036 pages 858-860	1-6

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
13 September 2016	21/09/2016

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1

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Claudio Palomo ET AL: "New Synthesis of a-Amino Acid N-Carboxy Anhydrides through Baeyer-Villiger Oxidation of a-Keto &Lactamst", J. Org. Chem. Ber. Dtsch. Chem. Ges.. Dtsch. Chem. Ges. J. P.; Winitz, M. Chemistry of the Amino Acids, 15 April 1994 (1994-04-15), pages 39-861, XP055236138, Retrieved from the Internet: URL:http://pubs.acs.org/doi/abs/10.1021/jo00090a033?journalCode=joceah&quickLinkVolume=59&quickLinkPage=3123&selectedTab=citation&volume=59 page 3124	1-6
A	EP 0 752 419 A2 (DEGUSSA [DE]) 8 January 1997 (1997-01-08) claim 1	1-6
A	WO 2005/095340 A1 (DEGUSSA [DE]; ROSSEN KAI [DE]; HOFFMANN ROLF [DE]; SARICH MARTIN [DE]) 13 October 2005 (2005-10-13) claim 1	1-6

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/EP2016/068347

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