Organic Process Research & Development

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Carry Over of Impurities: A Detailed Exemplification for Glycopyrrolate (NVA237)

Thomas Allmendinger,* Dominique Bixel, Adrian Clarke, Laura Di Geronimo, Jean-Wilfried Fredy,† Marco Manz, Elena Gavioli, Regine Wicky, Martin Schneider, Fabien J. Stauffert,‡ Markus Tibi, and Darko Valentekovic

Novartis Pharma AG, Technical Research and Development, CH-4002 Basel, Switzerland

Supporting Information

ABSTRACT: The original synthesis of glycopyrrolate (NVA237) was revised and shortened into an essentially one-pot process. Without isolating the intermediates, their purification became obsolete, thereby increasing the possibility of the carry over of impurities. For that reason, the actual, potential, and theoretical impurities of the starting materials cyclopentyl mandelic acid and 1-methyl-pyrrolidin-3-ol as well as byproducts which may occur during the synthesis were thoroughly investigated; furthermore, their transformation to possible impurities in the drug substance along the new synthetic route was performed to exclude them as actual impurities in the drug substance with certainty. The question is raised how detailed such investigation—which are fairly manageable for a simple product like glycopyrrolate—need to be.

1. INTRODUCTION

Glycopyrrolate is a well-known antagonist of muscarinic receptors and is on the market for the treatment of sialorrhea,[1] hyperhydrosis,[2] and overactive bladder and for presurgery treatment. Similar anticholinergic agents like the atropine analogues ipratropiumbromid and tiotropiumbromid[3] were recently introduced for asthma patients to treat chronic obstructive pulmonary disease (COPD). Novartis has developed glyocopyrrolate for this indication.[4]

The key intermediate for its synthesis is the ester **3** of cyclopentyl mandelic acid (CPMA, **1**) with 1-methyl pyrolidin-3-ol **2**. As a hydroxyacid, usual activations of **1**—e.g., as acid chloride—are not possible; following the original procedure patented in 1960,[5,6] such esters are therefore mainly prepared by transesterification[7] of methyl cyclopentylmandelate (MCPM) **1a**[8] and **2** in the presence of metallic sodium or sodium hydride (see Scheme 1). As a tertiary amine, the ester **3** can be purified by extraction of its salt **3x** into water to remove impurities followed by back extraction into an organic solvent.

The final step of the synthesis is comprised of the N-methylation of **3** using methyl bromide **4** to form the solid quartenary ammonium salt **5**. As **1** and **2** are applied as racemates, **5** is formed as a mixture of two pairs of enantiomers which are separated by final repeated recrystallization to get the desired higher melting RS/SR-diastereomer **6**.

Although the synthesis is easy from a chemical point of view, the scale up in the pilot and production facilities bears some drawbacks. The transesterification using metallic sodium or sodium hydride represents a severe safety problem. Furthermore, the handling of the intermediate **3** needs special attention as it is known to cause reversible exogenous psychosis when exposed to man,[9] and the loading of numerous vessels and tanks with solutions containing **3** or **3x** is potentially more hazardous. To cope with these HSE issues, Novartis came up with a shortened synthesis which will be described briefly in section 2.

Minimizing its handling by skipping isolation, purification, and analytical testing of the intermediate **3**, the new process becomes potentially more sensitive to impurities. The remainder of the article will therefore describe the detailed investigation of their so-called carry over. Sections 3 and 4 discuss the theoretical and potential impurities in starting materials **2** and **1**, respectively. Section 5 describes the preparation of some of these impurities as reference compounds, whereas section 6 is devoted to the question how these potential impurities would behave in the synthesis towards the drug substance. Section 7 describes the potential impurities which may occur due to side reactions in the conversion of **1** and **2** to final drug substance **6**, and section 8 tries to highlight some general ideas and to summarize the results in a single table.

Carry over studies as described are not only the basis for the setting of specifications for raw materials with respect to particular impurities but also contribute to the enhanced process and product understanding which are the basic tenets of Quality by Design (QbD),[10] as the quality of raw materials is one of the sources of variability during manufacturing and thus constitutes and limits the so-called (multidimensional) design space.[11]

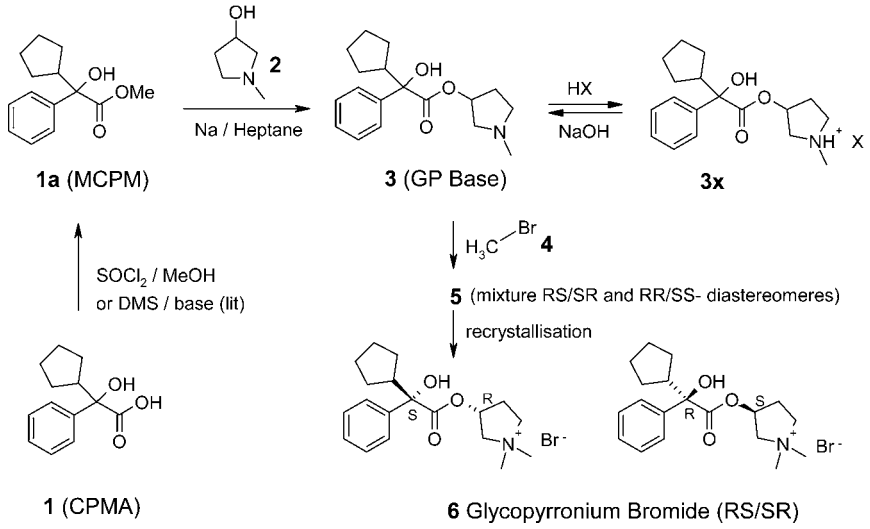
2. ONE-POT SYNTHESIS OF GLYCOPYRROLATE

For the reasons mentioned above, we looked for the direct esterification of acid **1** with the aminoalcohol **2**. Published conditions for similar starting materials using mixed anhydrides[12] were unsuccessful in our case, but the use of carbonyl diimidazole (CDI) gave much better results.[13] Although the imidazolide **7**—so far unknown—can be isolated as a solid compound, we performed this activation in situ (see Scheme

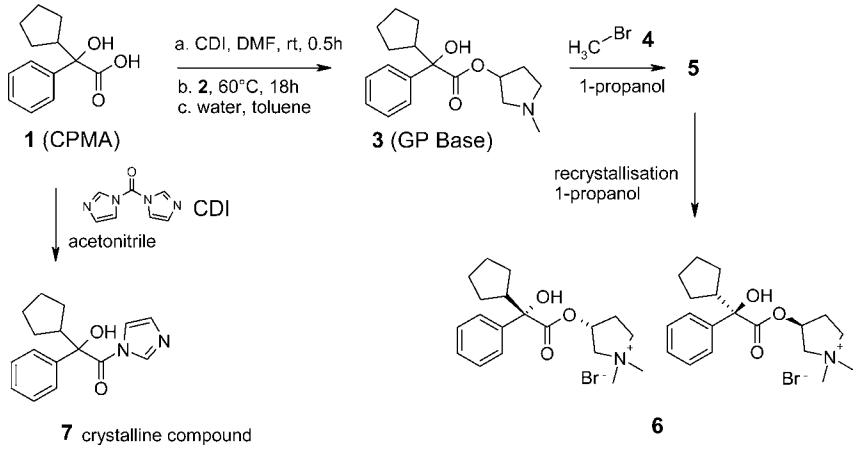
Received: July 4, 2012
Published: October 8, 2012

dx.doi.org/10.1021/op3001788 | Org. Process Res. Dev. 2012, 16, 1754–1769

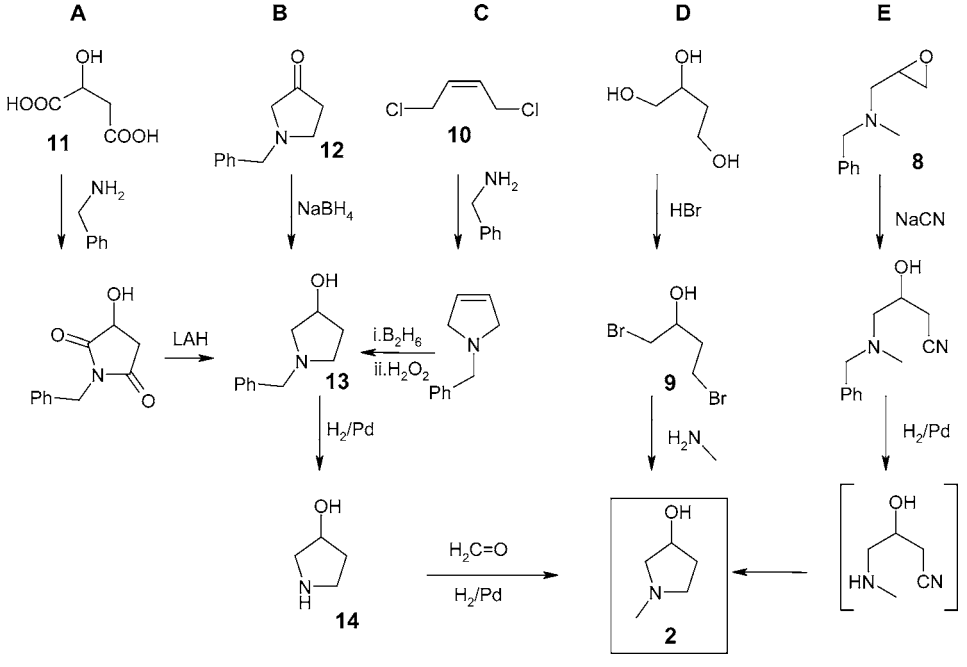
Scheme 1. Classical Synthesis of Glycopyrronium Bromide 6



Scheme 2. Improved Process for the Preparation of Glycopyrrolate 6



Scheme 3. Synthetic Pathways to Prepare 1-Methyl-pyrrolidin-3-ol 2



dx.doi.org/10.1021/op3001788 | Org. Process Res. Dev. 2012, 16, 1754−1769

2). Thus, **1** in DMF is treated with a slight excess of CDI at room temperature followed by the addition of **2**. The esterification is slow at room temperature, requiring three days for completion, but is accelerated by heating. After the aqueous quench, the product **3** is extracted into a water immisible organic solvent like toluene or TBME. Initially the purification process via **3x**[14] was applied, but after careful optimization of the esterification it was proved to be unnecessary. Thus, the organic extract of the hydrolyzed reaction mixture is just washed with water and can be directly used for the next step in the same vessel.[15]

For the quarternization reaction, nonpolar solvents like TBME and toluene are not well suited, and a solvent exchange is needed. Originally we followed the published process[16] using acetone from which the solid mixture **5** of both racemic diastereomers crystallizes in high yield. For the final recrystallization, we found 1-propanol as the solvent of choice to remove the undesired RR/SS-diastereomer. Thus, by switching to 1-propanol in the methylation reaction, still forming both diastereomers in solution, the crystallized crude product **5** was already highly enriched in the desired RS/SR-pair;[17] the final recrystallization became less laborious; and usually two more recrystallizations were sufficient to decrease the content of the undesired diastereomer (RR, SS) below 0.1%.

By skipping one step (**1** to **1a**), improving the esterification and telescoping the crude ester **3** directly into the methylation reaction,[18] followed by crystallization and recrystallization, the synthesis of glycopyrrolate became very short. The purification of the intermediate **3** is unnecessary as well as impossible,[19] and the fate of potential and actual impurities needs to be clarified in more detail.

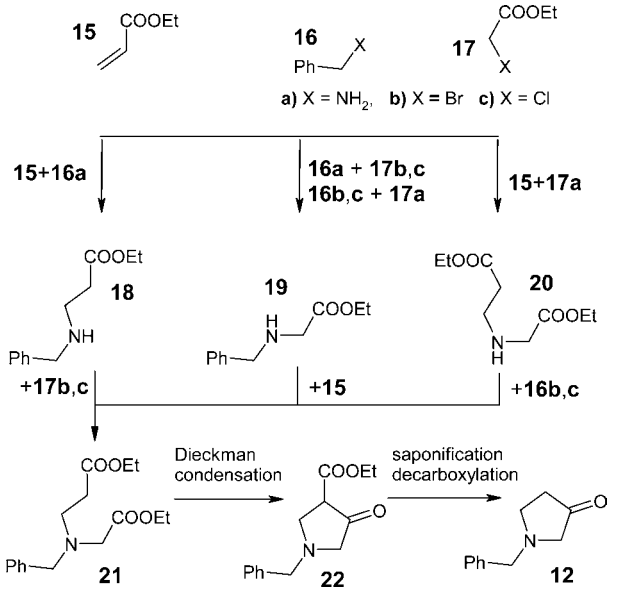
3. THEORETICAL AND POTENTIAL IMPURITIES IN 1-METHYLPYRROLIDIN-2-OL

For such a short synthesis, the suitability of **1** and **2** as regulatory starting materials may be questioned, and health authorities require detailed information about their precursors including release criteria, i.e., what are the potential and actual impurities and their accepted levels and how are these precursors transformed into **1** and **2**.

Scheme 3 depicts the published routes for the preparation of **2**.[20] Route E starts with a 3-carbon unit, the epoxide **8**, and adds one more carbon by the use of cyanide.[21] Final catalytic debenzylation and concurrent reductive cyclisation give **2**. Route D starts with a 4-carbon unit already.[22] Methylamine is alkylated with dibromobutanol **9**—obtained from butanetriol—to form **2**.[23] Routes A, B, and C have 1-benzyl-pyrrolidin-3-ol **13** as a common intermediate. Route C uses *cis*-1,4-dichlorobutene **10** as the 4-carbon unit which is reacted with benzylamine followed by hydroboration−oxidation.[24] Malic acid **11** is the precursor for route A which is cyclised with benzylamine followed by reduction,[25] and in route B, the aminoketone **12** is just reduced to get the aminoalcohol **13**.[26] Debenzylation to form **14** and reductive methylation complete the sequence to compound **2**.[27]

Alkylating agents like **8**, **9**, and **10** immediately attract our attention as potential genotoxic impurities. In addition, looking at its preparation more closely, **12** loses its innocence: among its precursors appear further alkylating agents like ethyl acrylate **15**, benzyl halides **16b/c**, and ethyl halo acetates **17b/c** (see Scheme 4).[26,28]

Scheme 4. Pathways to 1-Benzyl-pyrrolidin-3-on 12, Precursor of Route B



In view of patient safety, alkylating agents need to be strongly controlled as potential impurities in the drug substance but also already in the starting material. However, are the electrophilic alkylating agents not expected to be destroyed by the nucleophilic amine **2**? This was tested experimentally. Samples of **2** were mixed with small amounts of these reagents **8**, **9**, **9a**,[29] **10**, **15**, **16b/c**, and **17b/c**; in partly exothermic reactions, the alkylating agents disappear as can be seen by GC analyses,[30] and the quaternary ammonium[31] salts **23**−**29b** are formed and detected by HPLC-MS (see Scheme 5).[32] In an ocean of neat **2**, any small concentration of these alkylating agents will decline to zero in reactions following pseudo-first-order kinetics. They can thus be excluded from further consideration as potential impurities.

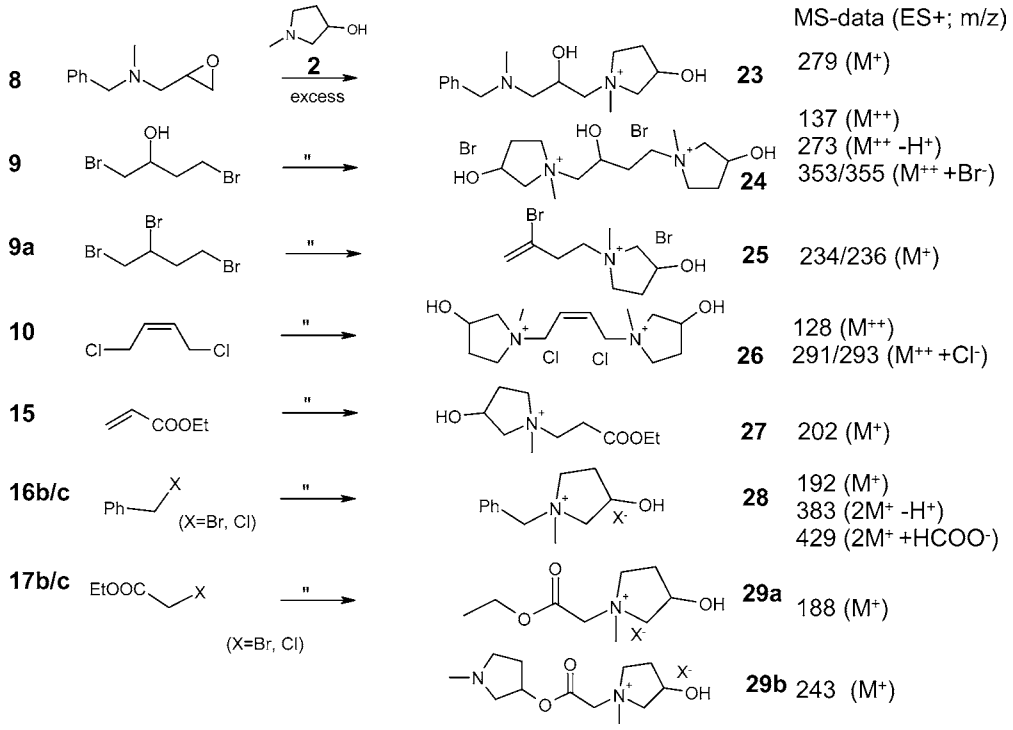
Revisiting Schemes 3 and 4, the compounds **13**, **14**, **16a**, **18**, **19**, and **20** remain as potential impurities bearing the functionality (OH or NH) to be carried on into the drug substance (see Scheme 6, top row). In addition, **30** and **31** are known byproducts of route D and derived from tribromobutane **9a** and dibromobutanol **9**, respectively.[33] Repeating the preparation of **2** by route D, the open chain diamine **32**[34] can be detected in the crude reaction mixture by GC, LC-MS, and ^{13}C NMR. An "isomer" of **2** was indicated by GC-MS whose structure was finally assigned to 3-methylamino-tetrahydrofuran **33**[35](see section 8). Furthermore, although requiring strong oxidizing agents or harsh conditions,[36] tertiary amines may form *N*-oxides; therefore, **34**—so far unknown—was prepared as a reference by reacting **2** with H_2O_2.

Finally, the piperidine derivative **35a** is a reasonable impurity in **13** when it is prepared according to route B (see Schemes 3 and 4): formed by double Michael addition of benzylamine **16a** to ethyl acrylate **15**,[37] the diester would follow the Dieckmann-cyclisation/hydrolyses/reduction sequence to get **35a**[38] subsequently transformed to **35b** and **35c**.

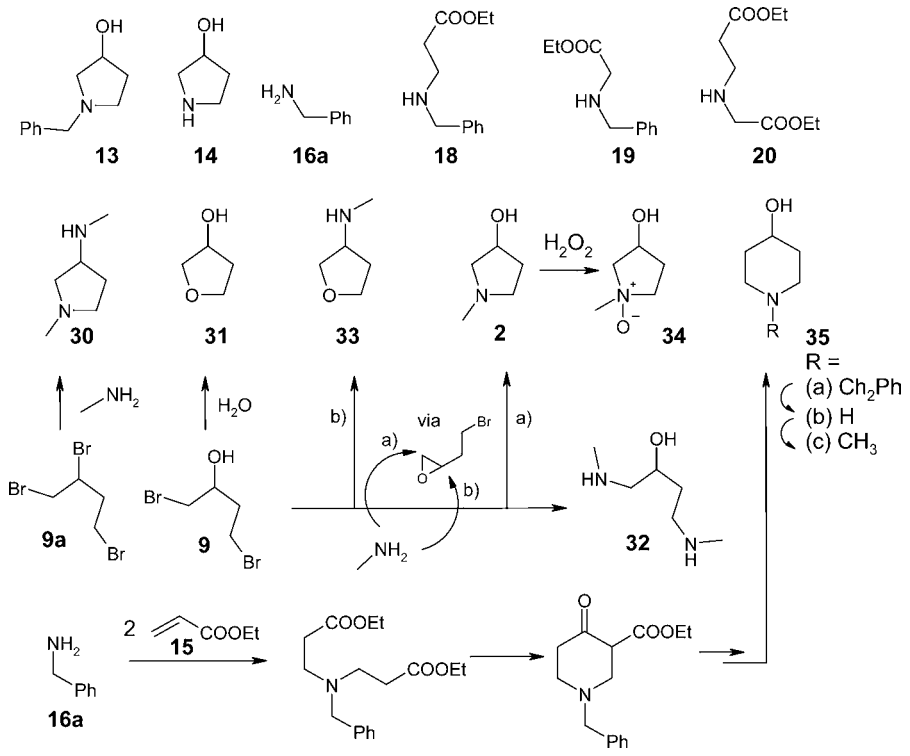
These 14 compounds[39] were therefore tested in the raw material control procedure for **2**.[40] The GC-method applied separates them all, and (if present) they would thus be identified and quantified as impurities in 1-methylpyrrolidin-3-

dx.doi.org/10.1021/op3001788 | Org. Process Res. Dev. 2012, 16, 1754−1769

Scheme 5. Alkylating Agents and Their Reaction with 2, MS-Data of Products



Scheme 6. Compounds Which Need to Be Regarded as Potential Byproducts in 2 and Their Formation



ol **2** even though some of the potential impurities have retention times very close to that of **2** (see Table 1).[41]

As 1-methyl-pyrrolidin-3-ol **2** is isolated and purified by fractional distillation, the likelihood of the presence of impurities is furthermore influenced by their boiling point (see Table 1, last column). From these data, only compounds **31** and **16a** (hydroxy-THF and benzylamine) and eventually pyrrolidin-3-ol **14** and 1-methyl-piperidin-4-ol **36c** are somehow possible to appear in the distillate. All others will be removed by distillation if at all present in the crude product of **2**. In fact, distillation trials with spiked samples revealed **30** and **33** to distill in the forerun, and **14** and **31** are collected in later distillation fractions. High boiling compounds like **13** and **32** remain completely in the distillation residue. The analyses of

dx.doi.org/10.1021/op3001788 | Org. Process Res. Dev. 2012, 16, 1754−1769

Table 1. Retention and Relative Retention Time as Well as Boiling Points of Potential Byproducts of 2

#	compound	Rt [min]	RRt	BP[a]
2	1-methyl-pyrrolidin-3-ol	3.67	1.00	74
13	1-benzylpyrrolidin-3-ol	7.29	1.99	143
14	pyrrolidin-3-ol	3.76	1.03	102
16a	benzylamine	4.69	1.28	90
18	ethyl 3-benzylamino-propionate	7.60	2.07	190
19	*N*-benzylglycin ethylester	7.20	1.96	150
20	ethyl 3-(ethoxycarbonylmethyl-amino)-propionate	6.60	1.80	190
30	*N,N′*-dimethyl-3-aminopyrrolidine	4.08	1.11	45
31	3-hydroxy-tetrahydrofuran	3.39	0.92	80
32	1,4-bismethylamino-butan-2-ol	5.37	1.46	>100[b]
33	methyl-(tetrahydro-furan-3-yl)-amine	3.82	1.04	40
34	1-methyl-1-oxy-pyrrolidin-3-ol	decomposition		
35a	1-benzyl-piperidin-4-ol	7.77	2.12	185
35b	piperidin-4-ol	4.44	1.21	112
35c	1-methyl-piperidin-4-ol	4.47	1.22	103

[a]Boiling point from SciFinder, in °C at 15 Torr. [b]Compound **32** so far unknown, see Supporting Information, measured bp 92 °C/1 mbar.

selected batches of **2** contain indeed up to 0.5% of **14** or **33**[42] and **31**.

4. THEORETICAL, POTENTIAL, AND ACTUAL IMPURITIES IN CPMA

Repeating the exercise for the other starting material CPMA **1** turned out to be exciting and interesting. CPMA **1** was first described in 1952[43] using a method going back to Grignard himself (1902).[44] Ethyl phenylglyoxylate **36b** is reacted with cyclopentyl magnesium bromide to form the ester **1b** which is hydrolyzed and purified (Scheme 7A). Slight modifications are the use of phenylglyoxylic acid **36** (B)[45] and cyclopenta***dien***yl magnesium bromide which requires an additional hydrogenation step (C).[46] Using an inverse approach (D), the phenyl-Grignard reagent is reacted with cyclopentyl-glyoxylic acid ester.[47] More sophisticated methods use dichloromethane and cyclopentyl phenyl acetic acid which are deprotonated with BuLi at a (very) low temperature and reacted with phenyl-cyclopentyl ketone[48] and oxygen,[49] respectively. These latter methods E and F are not easily scaled up, bear severe safety issues, and are most likely not used by manufacturers. The commercially applied methods might thus be A–D, also justified by the fact that these procedures are claimed in patents.[50]

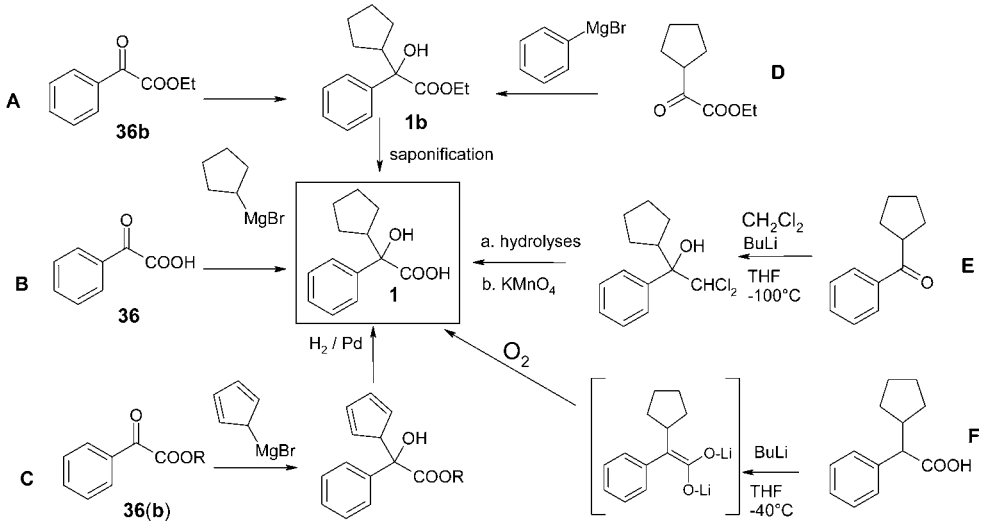
According to the published descriptions, the yields of **1** ranging from 28 to 53% are surprisingly low.[51] To understand it better and to learn about possible side products,[52] we looked into the reaction of methyl and ethyl phenylglyoxylate **36n** and phenylglyoxylic acid **36** with cyclopentyl magnesium halide more closely. Scheme 8 shows the product distribution of crude **1** prepared by the original Grignard approaches (A and B).[53] The desired **1** and phenyl glyoxylic acid **36**, formally unreacted starting material or its product of hydrolyses,[54] are easily explained. A major byproduct is mandelic acid **37** which is also easily rationalized as the product of reduction by *β*-hydride transfer from the Grignard reagent. Cyclopentylidene phenyl acetic acid **42** is the product of water elimination from **1** or **1n** occurring under (Lewis-) acidic conditions.[55]

The cyclopentylether **38** is reported once as the product of an "anomalous reaction of cyclopentylmagnesium chloride", the attack of the Grignard reagent at the carbonyl-oxygen.[56] Although benzoic acid **39** might be derived from phenylglyoxylic acid **36** by decarbonylation, this reaction is reported to occur only under oxidative and photolytic conditions.[57] Therefore, **39** and the unsaturated ("oxidized") compounds **40** and **41** call for a separate explanation. This is given by the frequently discussed radical pathway for the Grignard formation and reaction.[58] Scheme 9 gives a summary of the proposed ET (electron transfer) mechanism to explain essentially all byproducts.

The intermediacy of radicals and radical-anions explains the formation of dimers like **43n** and **44n** which can be detected in the crude esters by LC-MS analyses. Compounds of that kind are described as polymerisation initiators and are indeed prepared from alkyl phenylglyoxylates **36n** by the reaction with alkyl or silylhalides and magnesium.[59] 1,2-Diphenyl tartraric acid **43** obtained after saponification of **43n** is an unstable compound and is known to decompose into **36**, **37**, and **39**.[60]

Radical intermediates also explain the formation of unsaturated compounds **40** and **41** by H-atom abstraction

Scheme 7. Published Synthetic Pathways to CPMA 1



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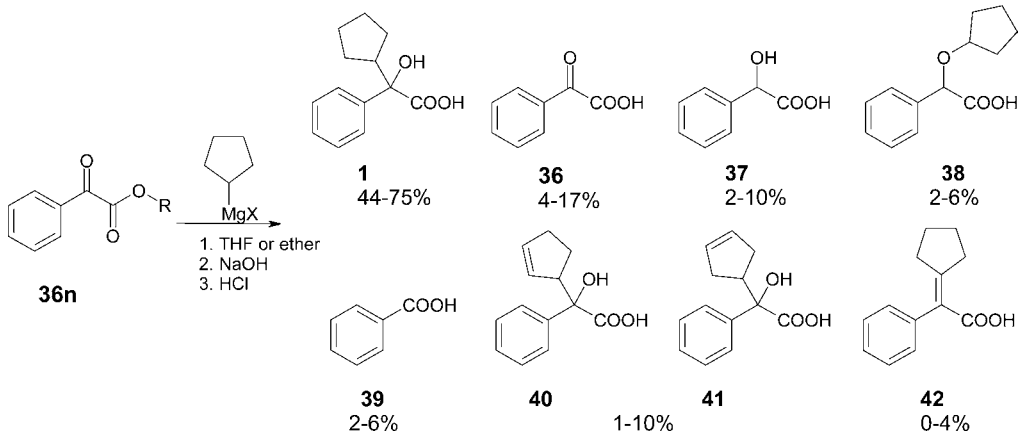
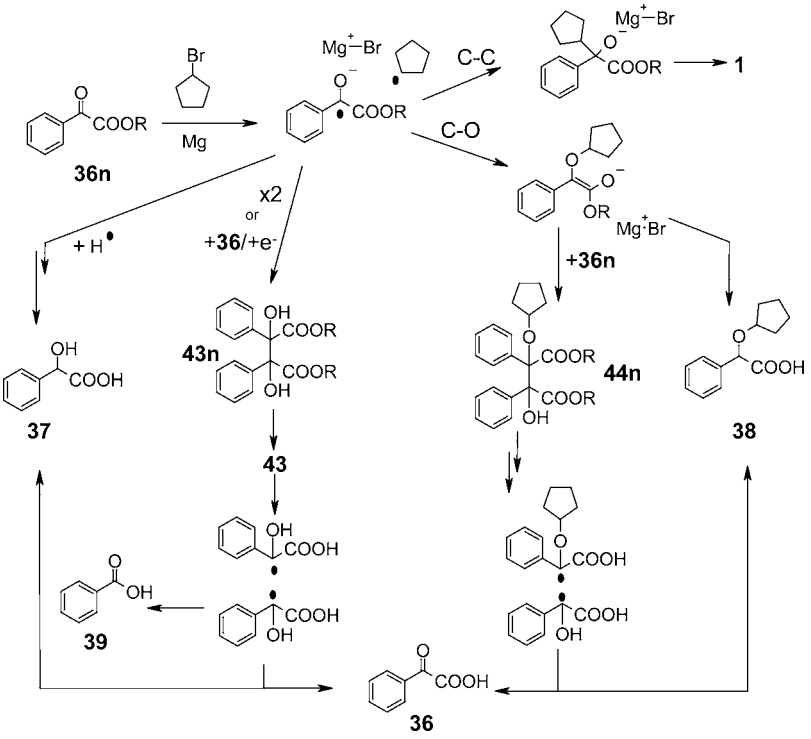
Scheme 8. Product Distribution of Crude 1 Prepared by Method A

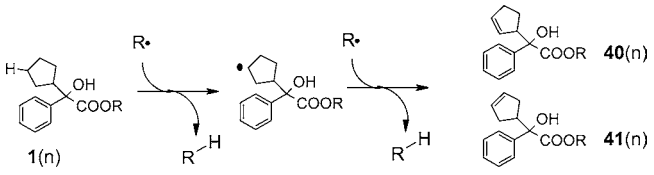


Scheme 9. Proposed Mechanism to Account for the Products and Byproducts



from **1** or its ester (see Scheme 10).[61] These compounds are not explicitly mentioned in the literature; one patent, claiming an improved process for the preparation of glycopyrrolate **6**, describes a hydrogenation step after the formation of the GP-base **3** to reconvert its unsaturated byproducts.[62]

Scheme 10. Proposed Origin of 40 and 41



All the byproducts found in crude batches of **1** are thus being explained and are potential byproducts, although their content is tremendously diminished by recrystallization as can be seen by the purity of a number of CPMA (**1**) samples which we gathered from the worldwide market (see Table 2 and Supporting Information).[63] One impurity, variable in the samples between 0 and 0.8%, was unknown. Its content correlates somehow with the content of organic-bound chlorine, a value which is not routinely determined. The byproduct in question was thus confirmed by HPLC-MS to be a chlorine-containing compound. After isolation and independent preparation (see section 5), its structure was verified as the *p*-chloro analogue **45**.[64]

5. INDEPENDENT PREPARATION OF CPMA BYPRODUCTS

To make the impurities in question available in gram quantities as reference compounds and precursors for the subsequent transformations, a facile route for their preparation is essential. This chapter gives descriptions for the preparation of **38**, **40**, **42**, and **45**.

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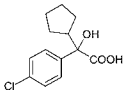
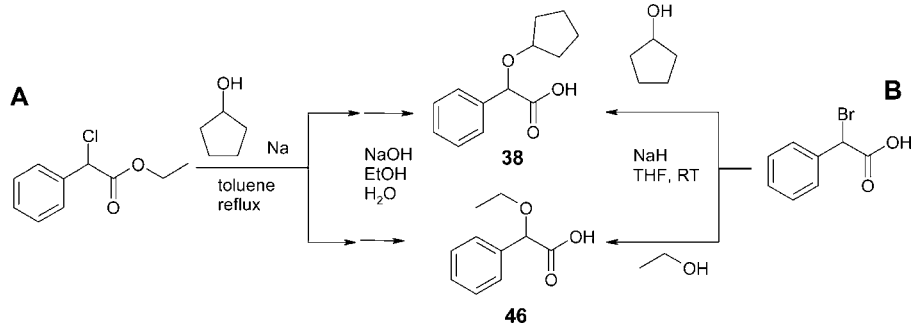
Table 2. Composition of CPMA (1) Obtained from Different Suppliers (a–k) (HPLC, area %) and the Content of Organic-Bound Chlorine (In % by Elemental Analyses)

	Suppliers of CPMA (**1**)										
(by) product	a	b	c	d	e	f	g	h	i	j	k
36	-	-	-	-	-	-	-	-	-	-	-
37	-	-	0.03	-	-	0.01	0.02	0.02	-	0.01	-
38	-	-	-	-	0.31	0.30	0.29	0.25	-	-	-
39	-	-	-	0.02	0.02	0.02	-	0.03	-	-	-
40	0.18	0.06	0.06	0.16	0.13	0.12	0.09	0.13	0.07	0.02	0.04
41	0.38	0.15	0.03	0.42	0.06	0.06	0.03	0.07	0.15		0.02
CPMA **1**	**98.74**	**97.61**	**99.83**	**98.46**	**98.67**	**98.68**	**99.24**	**98.61**	**99.40**	**97.87**	**99.88**
42	0.04	0.63	-	0.08	0.02	0.03	-	0.04	-	-	-
toluene	0.20	0.12	0.02	0.26	0.17	0.16	0.13	0.14	0.19	1.84	0.02
45	**0.37**	**0.80**	-	**0.33**	0.07	0.06	-	0.07	**0.16**	-	-
sum of unknown	0.1	0.6	0.0	0.3	0.6	0.6	0.2	0.6	0.0	0.3	0.1
Org. bound chlorine	**0.09**	**0.18**	0.01	**0.13**	0.01	0.01	0.01	0.01	**0.05**	0.00	0.00

45  Cl-CPMA

Scheme 11. Preparation of 38 According to a Published (A) and a New Process (B)

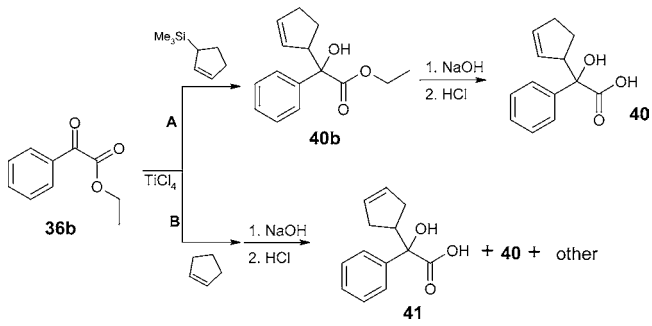


The independent preparation of *O*-cyclopentyl-mandelic acid (cyclopentyloxy-phenyl-acetic acid), **38**, was described in the paper mentioning its formation by "anomalous" Grignard reaction;[54] however, following the description gives a quite impure material containing the corresponding ethoxy compound **46** (see Scheme 11, A).[65] Therefore, a new and much simpler method was established (B) by reacting 2-bromo-2-phenylacetic acid with cyclopentanol in THF after addition of sodium hydride to form **38** selectively. The ethoxy compound **46** is also accessible when cyclopentanol is replaced by ethanol.

The isomers of the unsaturated impurities, the cyclopent**en**yl mandelic acids **40** and **41**, were isolated from impure samples (e.g., sample **a** in Table 2) by preparative HPLC. For the ester **40b**, a literature method is described by reacting 3-TMS-cyclopent-1-ene with ethyl phenylglyoxylate **36b**,[66] and the process was applied successfully and completed to the acid **40** (see Scheme 12 route A). In analogy to the clean reaction of cyclohexene with **36b** to form 2-(cyclohexen-3-yl) phenyl-glyoxylic acid esters,[67] the reaction with cyclopentene and subsequent saponification was performed but gave a complex mixture containing **40** and **41** in the range of only 10–20% (see Scheme 12 route B).[68,69]

Cyclopentylidene-phenyl-acetic acid **42** is also described in the literature (see Scheme 13, A and B),[70] but again a much simpler approach is available (C): CPMA **1** is just refluxed in 6 N HCl for two days to form the dehydrated **42** which

Scheme 12. Preparation of 40 and 41 by Lewis Acid Catalyzed Alkenylations

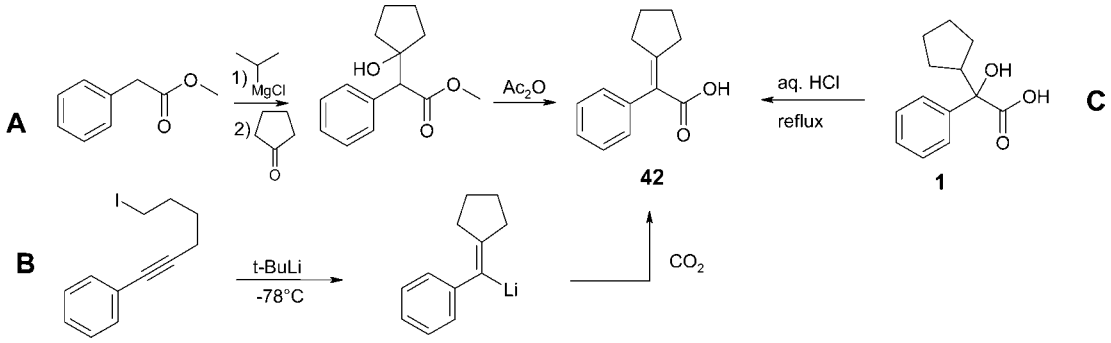


crystallizes upon cooling and can be purified by recrystallization.

By analogy to the preparation of CPMA itself, we assume Cl-CPMA **45** to stem from *p*-Cl-phenylglyoxylic acid or its ester **47b** as an impurity of the starting material phenylglyoxylic acid **36** or its ester **36n**. In fact, the reaction of cyclopentyl-magnesium bromide with **47** is described to yield 12% of **45**,[71] but initial attempts to repeat this conversion failed (see Scheme 14). For the time being, we prepared **45** as a reference compound by the inverse process from cyclopentyl-glyoxylic acid ester using the *p*-Cl-phenyl-Grignard reagent.

dx.doi.org/10.1021/op3001788 | *Org. Process Res. Dev.* 2012, 16, 1754–1769

Scheme 13. Literature (A, B) and Simplified Process (C) to Obtain 42



Scheme 14. Preparation of Cl-CPMA 45

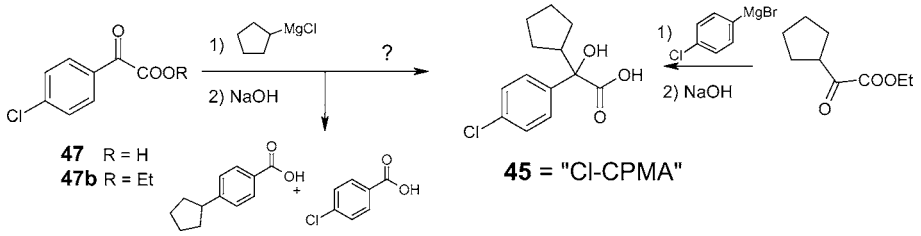
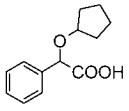
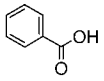
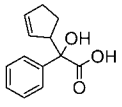
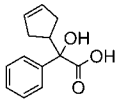
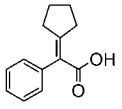
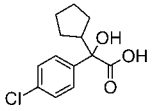


Table 3. Potential Impurities in Starting Material 1 and the Corresponding Products of Carry over onto the Drug Substance Step and Their Depletion Factor, DF, during One Crystallization

Potential impurity in **1**	Corresponding pot. impurity in DS **6**.			RRT	Content in **6** (spiking experiment)	Approx. DF
38		**48**	a)	1.09 1.10	≤0.04%	>11
39		**49**	a)	0.49	0.04%	24
40		**50**	b)	*0.86*	*0.04% in **6** (0.07% in **5**)*	*2**
41		**51**	b)	*0.90*	*0.24% in **6** (0.28% in **5**)*	*1**
42		**52**	a)	1.20	0.06%	34
45		**53**	a) b)	*1.26*	*0.98% in **6** (0.92% in **5**)*	*0.94**

[a)]Prepared from starting impurity by its activation with CDI and **2**, followed by methylation. [b)]Isolated from drug substance batch. *Data from a drug substance batch which was prepared from a precursor **1** contaminated with 0.18%, 0.38%, and 0.37% of **40**, **41**, and **45**, respectively (see Table 2, "batch **a**").

6. CARRY OVER AND DEPLETION OF POSSIBLE BYPRODUCTS IN STARTING MATERIALS

We have discussed the possible byproducts of starting materials **1** and **2**; if present, they would further react according to the conditions of the desired reactions. Tables 3 and 4 list the potential byproducts **48**–**62** which can thus be expected and which have been synthesized or isolated (see Supporting Information). With these reference compounds in hand, their separation from the drug substance using the given HPLC conditions could be confirmed (see relative retention times,

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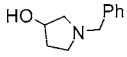
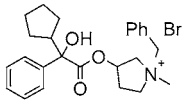
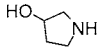
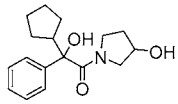
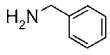
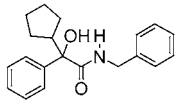
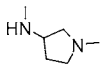
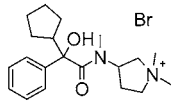
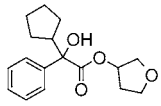
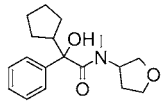
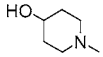
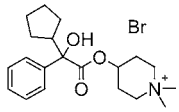
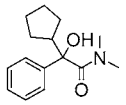
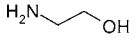
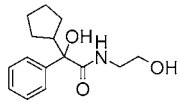
Table 4. Potential Impurities in Starting Material 2 and the Corresponding Products of Carry over onto the Drug Substance Step and Their Depletion Factor, DF, during One Crystallization

Potential impurity in **2**		Corresponding pot. impurity in DS **6**.			RRT	Content in **6** (spiking experiment)	Appr. DF
13			**54**	c)	1.34	<0.015% <0.015%	>5 >6
14			**55**	d)	1.05 1.06	0.04% <0.015%	2 >4
16a			**56**	d)	1.74	<0.05%	>37
30			**57**	e)	0.99 1.03	0.025% 0.052% *	17 10
31			**58**	d)	1.67	<0.015%	>9
33			**59**	d)	1.48 1.95	<0.05%	>2
35c			**60**	e) f)	1.07	0.015%	69
g)			**61**	d)	1.53	<0.015%	>170
h)			**62**	d)	1.09	≤0.04%	>11

[c] Prepared from **3** and benzyl bromide. [d] Prepared from starting impurity and **1** after its activation with CDI. [e] Prepared from starting impurity and **1**, followed by methylation. [f] Compound **60** is described.[78] [g] Dimethylamine is a potential impurity in DMF, the solvent for the reaction of **1** to **3**. [h] Ethanolamine is used to destroy methylbromide **4** in the mother liquor of **5** in a waste reactor; if by mistake it comes into contact with product or intermediates **3**, **5**, **6**, or **7**, the corresponding amide **61** would be formed. * LC-MS detection and ion-specific quantification.

RRT),[72] and their absence in the drug substance can be verified with certainty from an analytical point of view.[73] Furthermore, from a chemical point of view the depletion of these impurities during the crystallization of the drug substance is important information. We define the depletion factor as the ratio of the content of the byproduct before and after recrystallization, DF = c^0/c^K.

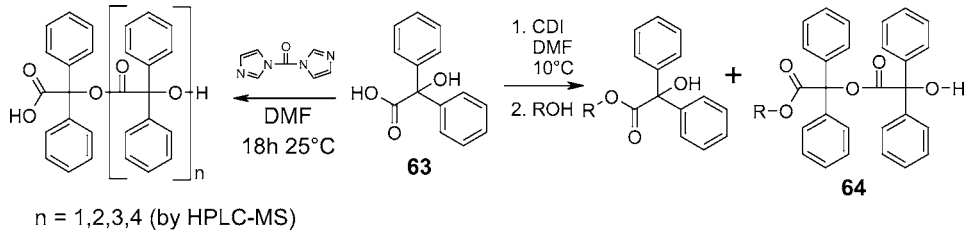
For the impurities **50**, **51**, and **53**, we derived this information from the analyses of batches where these impurities were present.[74] For the potential impurities **48**, **49**, **52**, and **54**–**62**, we determined this depletion factor from spiking experiments. The impurities were added in quantities of about 1% to a typical final crystallization experiment of the drug substance. The samples of compound **6** after *one* recrystallization were analyzed by HPLC to quantify the content of the byproduct. This is given in Tables 3 and 4, showing that all tested additives appear to be <0.1% (a).[75] The depletion factors (DF) are calculated by eq 1[76] and are also given in Tables 3 and 4.

$$\mathrm{DF} = \frac{c^0}{c^K} = y + (1 - y)\frac{c^{ML}}{c^K} \quad (1)$$

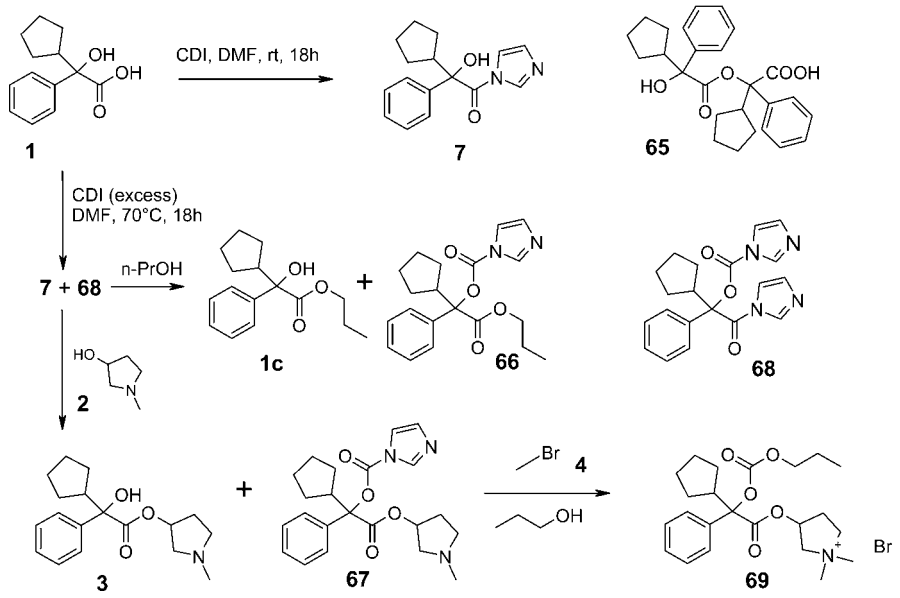
Most of the potential impurities show a depletion factor (DF) >10, indicating their content will drop by a factor of >100 when the DS is recrystallized twice. The three compounds **50**, **51**, and **53** already mentioned have much smaller depletion factors (or do not deplete at all) and thus appear as critical impurities. Their precursors **40**, **41**, and **45** must be carefully limited in the starting materials to <0.05%. One of the isomers of compounds **55** and **59** also depletes with a factor of 2 only. Considering that its precursors **14** and **33** are splitting into two isomers of compound **55** (factor 2), the more critical isomer is

dx.doi.org/10.1021/op3001788 | Org. Process Res. Dev. 2012, 16, 1754–1769

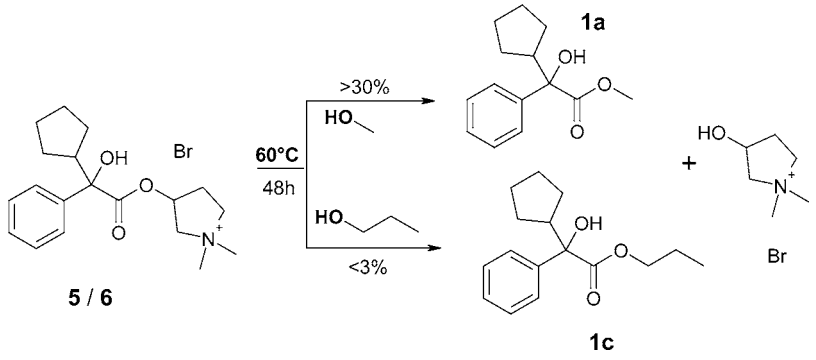
Scheme 15. Esterification of Benzilic Acid with CDI and Alcohols



Scheme 16. Reactions and Side Reaction of CPMA (1) with CDI and Alcohols, Carry Over Products 66, 67, and 69



Scheme 17. Decomposition of Glycopyrrolate 6 by Heating in Alcohols to Form the Corresponding Esters 1n



depleting with a factor of 2, and the crystallization is performed 3 times,[77] the overall depletion factors are also as high as 16. One of the isomers of **57** does not analytically separate from **6**. The depletion, however, is well justified by HPLC-MS analyses of the crystallized **6** and its mother liquor from the respective spiking experiment; the mother liquor shows under the peak of the drug substance (M^+ = 318) also the peak of the spiked impurity (M^+ = 331), whereas in the chromatogram of the crystallized material only M^+ = 318 can be detected. The depletion factor is high (>10) as can be determined by taking the responses for the extracted masses (see Supporting Information).

7. SIDE REACTIONS DURING THE SYNTHESIS TO THE FINAL DRUG SUBSTANCE

The problematic esterification of hydroxy acids after their activation was already mentioned (vide supra). We became aware of this during the work on esters of benzilic acid **63**, a starting material very similar to **1**. Reacting **63** and CDI in DMF at room temperature, the formation of oligoesters is quite fast and complete as revealed by HPLC-MS (see Scheme 15). When the reaction is performed at lower temperature and completed by the addition of an alcohol, the formation of dimeric esters **64** can be largely suppressed.[79]

With this experience in mind, we were pleased to notice that the esterification of CPMA (**1**) was much cleaner. Applying room-temperature conditions, the easily formed imidazolide **7** is stable (see Scheme 2), and the dimerization of the starting

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acid (to form **65**) as well as the reaction with added alcohols (like **2**) is slow;[80] they require higher temperature in contrary to benzilic acid. Under the conditions depicted in Scheme 2,[81] **3** is cleanly formed, and dimeric acids and esters cannot be detected by HPLC-MS.

The fairly long reaction time at 60 °C forced us to investigate possible side reaction of **1**. Therefore, CPMA **1** was reacted with excess CDI at 70 °C for 24 h followed by the addition of 1-propanol or **2** (see Scheme 16). In both cases, along with the expected and desired esters **1c** and **3**, the ester-carbamates **66** and **67** could be isolated as byproducts which must have derived from an intermediate imidazolide-carbamate **68**.

Thus, the ester carbamate **67** is a potential impurity in the intermediate **3**. **67**, isolated from the stress experiment, was subjected to a solution of methyl bromide **4** in propanol to form besides **3** and **5** a new compound which was identified by HPLC-MS as the quaternary salt **69** containing a carbonate functionality; when **5** is crystallized in the presence of this compound (as a crude reaction mixture), **69** remains in the mother liquor.

During the solvent screening for the final recrystallization, methanol was tested as well. However, heating solutions of the drug substance **6** in methanol showed its instability; within 4, 24, and 48 h at 60 °C, 13, 27, and 34%, respectively, of the methylester **1a** is formed (see Scheme 17).[82] In 1-propanol this degradation is much less pronounced: after 48 h at 60 °C, less than 3% of the drug substance was converted to the propylester **1c**.[83] This ester remains completely in the mother liquor, when the product **6** is crystallized by cooling.[84]

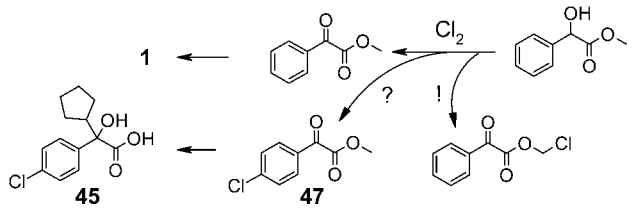
8. FURTHER DISCUSSION

Carry over of byproducts becomes an important task during the development of drug substances to achieve and secure high drug quality and to make sure that possible impurities are not overlooked. Glycopyrrolate used for the current study is a relatively simple molecule with only two starting materials. The investigation is based on the following questions:

(a) What are the actual and potential byproducts of the starting materials **1** and **2**?
(b) Are the analytical test methods for these raw materials selective to separate these (potential) impurities, which can thus be limited by specifications?
(c) What would be the fate of these impurities if present throughout the conversion of the starting materials to the drug substance, and would the corresponding derivatives be detectable during the analyses of the drug substance?
(d) What are potential side reactions during the conversion of the starting materials to the drug substance?

(a) To get a hold on *actual* byproducts requires the analyses of samples from diverse sources and includes multiple analytical techniques like LC- and GC-MS, semipreparative separation, isolation, and structure elucidation. Extremely helpful are vendors not only disclosing their route of manufacture but also supplying samples of crude products, mother liquors, or distillation fractions and residues.[85] On the other hand, *potential* byproducts of starting materials are just imagined in view of their synthetic origins which rely on literature and/or vendor information. However, a few impurities might not easily be guessed from the synthetic path from which the main compounds are produced. For example, Cl-CPMA (**45**) would not have come into our mind if not found in samples of **1**, or its derivative (**53**) in the drug substance. To imagine this, one would retro-synthetically need to go back one step further (see Scheme 18): CPMA ⇒ Phenylglyoxylate ⇒ **Mandelic acid.**

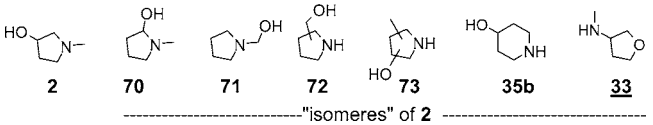
Scheme 18. Proposed Origin of Chlorinated Byproducts in CPMA (1)



Chlorinated byproducts are mentioned in the case that oxidation of mandelic acid is performed with elemental chlorine;[86] aromatic substitution is not unlikely forming *p*-chloro-phenylglyoxylate **47**.

Another example is the "isomer" of **2**, which was anticipated by the results of GC-MS analyses of a number of samples; however, what could be an isomer of 1-methyl-pyrrolidin-3-ol **2** (see Chart 1)? 1-Methyl-pyrrolidin-2-ol **70** or pyrrolidine-1-, 2-,

Chart 1. 1-Methyl-pyrrolidin-3-ol 2 and Possible "Isomeres"

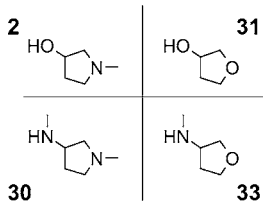


or 3-methanols, **71**, **72**, or one of the numerous *n*-methyl-pyrrolidin-*m*-ols (*n* = 2,3; *m* = 2,3,4,5) **73**? Although most compounds are described and accessible,[87] all of them do not make sense as a byproduct in **2** according to the diverse preparation methods. So, it is not a pyrrolidine derivative, and the ring homologue **35b** was ruled out already.[88]

We uncovered the structure after analyzing early distillation fractions of **2** prepared according to route D (see Scheme 3) where this isomer is enriched. Taking ^{13}C NMR spectra, the signals of an additional small 5-carbon unit matched quite well with the calculated spectra[a] of a compound where O and N in **2** are exchanged to constitute 3-methylamino-THF [methyl-(tetrahydrofuran-3-yl)-amine] **33**. Finally, repeated distillation of this fraction brought about sufficient material to confirm the structure, which is reasonable also from a mechanistic point of view;[35] **33** is also prepared independently from THF-3-carboxylic acid by Curtius degradation.[90]

Just for curiosity, with **33**, the "square" of 3-hydroxy or 3-methylamino-substituted 1-methyl-pyrrolidines and tetrahydrofurans span by **2**, **30**, **31**, and **33** is complete (see Chart 2); does the missing edge of a square give the hint to the answer of

Chart 2. Square of 3-Substituted 5-Membered Heterocycles Accessed as (By)products Following Route D (see Scheme 3)



dx.doi.org/10.1021/op3001788 | Org. Process Res. Dev. 2012, 16, 1754−1769

the question for the isomer? All four compounds appear as (by)products when following route D for the preparation of **2** (see Scheme 3).

(b) To test the specificity of the analytical raw material control procedure, all the potential impurities need to be available. In our case a lot of them were reported in the literature, and quite a few of them are claimed to be commercially available according to SciFinder data.[91] Others were prepared independently. With these compounds in hand, the analytical comparison and spiking was easy (see Tables 1 and 2).

(c) These impurities just need to be taken along the conditions of the main reaction[92] to get the corresponding derivative of the next steps. We considered all byproducts[93] which match the functional groups of the starting materials, acids like in **1** and alcohols (and also amines) in analogy to **2** which would undergo esterification (or amide formation) with **2** or **1**, respectively. The products of these transformations constitute potential byproducts in the drug substance and are used as reference compounds to test the specificity of the DS-analytical method. In addition, spiking gives a hint for the depletion capability of the final purification step, in most cases crystallization.

(d) Achieving conversions with high yield and with a minimal degree of side reactions is the duty of chemical development. Having found the final route and conditions, it is fairly common to take a thorough look into the robustness of the process: what happens if something goes wrong or a parameter is slightly changed? We call that quality risk analyses (QRA) and apply that systematically in all our projects. In addition, running the reactions under stressed conditions—resulting eventually in an out of specification result—highlights the direction in which the reaction is deviating and for what instance/byproduct one needs to look in cases of only slight modifications.

To summarize, the criteria to exclude potential impurities in the drug substance with certainty are: (1) separation from the drug substance in the HPLC-release method, (2) depletion during the drug substance purification process, and (3) exclude or limit their precursors as impurities in the starting materials. For the potential impurities of glycopyrrolate (for structures see Tables 3 and 4 and Schemes 16 and 17), the following matrix can be established (see Table 5).

We consider one of these criteria to be sufficient, but individually two or all may apply. In fact, 9 out of 13 potential impurities in glycopyrrolate stemming from potential by-products in starting materials fulfill all criteria, and for the other, 2 out of 3 criteria are valid.

Table 5. Potential Impurities in Drugs Substance and Their Precursors in the Starting Materials and Notification if They Fulfill the Criteria to Be Realized during Analytics or Depleted during Purification

Criterion *	Potential impurity in drug substance																
	48	**49**	**50**	**51**	**52**	**53**	**54**	**55**	**56**	**57**	**58**	**59**	**60**	**61**	**62**	**69**	**1c**
1	+	+	+	+	+	+	+	+	+	-	+	+	+	+	+	-	-
2	+	+	-	-	+	-	+	+	+	+	+	+	+	+	+	+	+
3	-	+	+	+	+	+	+	+	+	+	+	+	+	n.a.	n.a.	n.a.	n.a.
	38	**39**	**40**	**41**	**42**	**45**	**13**	**14**	**16a**	**30**	**31**	**33**	**35c**	**Imp. from side reactions**			
	Pot. imp. in **1** or **2** as precursor of pot. DS-impurity																
	1						**2**										

*1. Detection in DS-HPLC. 2. Depletion during DS-recrystallization. 3. Detection of corresponding precursor in starting material.

For example, methyl-(1-methyl-pyrrolidin-3-yl)-amine **30** is controlled on the level of the starting material by its separation from **2** during GC analyses, but the corresponding amide analogue **57** does not separate from the drug substance **6** in the HPLC. An experiment for the drug substance crystallization where **57** was spiked showed the depletion of this potential impurity when samples of crystallisate and mother liquor are analyzed by HPLC and MS-detection (see Supporting Information).

50, **51**, and **53** (detectable by HPLC and—if present—causing an out of specification result) do not deplete during the drug substance crystallization. As their precursors **40**, **41**, and **45** have baseline separation from **1**, they can easily be limited to <0.05%.

48 is separated and detected in the drug substance release, and it was shown to be depleted during crystallization; however, its precursor **38** does not fulfill criterion 3, and it is not separating from **1** in the raw material HPLC method. As a kind of fourth criterion, it was shown that **38** is depleting during the purification of **1** by recrystallization (as verified by an alternative HPLC-method, see Table 6 in the Supporting Information).

There are four potential impurities, **61**, **62**, **69**, and **1c**, due to possible side reactions which fulfill at least 1 out of 2 remaining criteria. CPMA propylesters **1c** and **69** are not detected in the HPLC method (UV);[94] their depletion is justified using another HPLC and/or detection method.

Altogether interesting journeys are in sight; however, the effort increases almost exponentially with the number of starting materials to be considered. Limitations are possible by focusing only on the last chemical steps, from the so-called API starting materials onwards. Additionally, we still know (or hope) that scientific reasoning is a valuable handle to answer the authorities' request "show me". In view of coming paradigms (QbD, PAT, continuous manufacturing, real time release), a thorough calibration will become even more evident.

9. SUMMARY

For glycopyrrolate (NVA237), a new one-pot process was established to avoid the handling of dangerous reagents and of highly active intermediates. As there is no isolated intermediate anymore which could be analyzed and if needed purified, the carry over of potential impurities was thoroughly investigated. For the starting materials, cyclopentyl mandelic acid **1** and 1-methyl-3-pyrrolidinol **2**, the potential byproducts as a result of their synthetic approaches have been discussed; they were purchased or prepared and analyzed according to the raw material testing instruction to show if they separate from the starting materials **1** or **2** and hence would be detected if present. An individual study showed that all alkylating agents which are used during the preparation of **2** and which are commonly regarded as carcinogenic compounds cannot be present in **2**; as impurities they would react with the tertiary amine functionality to form quaternary ammonium salts. Remaining potential impurities in starting materials were reacted further along the synthetic route, and the corresponding carry over byproducts were analyzed according to the drug substance release procedure to show their separation from glycopyrrolate. Experimentally it was shown that their content is diminished during the final recrystallizations except the unsaturated analogues **50** and **51** and the *p*-chloro-substituted **53**; thus, their precursors **40**, **41**, and **45**, respectively, need to be limited in the raw material **1**. In a last part of the study, the

reaction for the preparation of the intermediate **3** was investigated for possible side reactions and side products. The reaction appears to be very robust, and only one compound was shown to be formed under stressed conditions beside the propyl cyclopentylmandelate **1c** as a degradation product of the DS during the final crystallization; this degradation is slow, and the degradation product remains in solution. All potential byproducts fulfill at least one of two or three criteria to justify their absence.

■ ASSOCIATED CONTENT

Supporting Information

Full experimental detail and product characterisation. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Telephone: +41 (0)61 6968225. Fax: +41(0)61 6962711. E-mail: thomas.allmendinger@novartis.com.

Present Addresses

[†]UPMC Paris, Institut Parisien de Chimie Moléculaire.

[‡]Laboratoire de Synthèse Organique et Molécules Bioactives, Université de Strasbourg et CNRS (UMR 7509) Ecole Européenne de Chimie, Polymères et Matériaux.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We thank in particular Dr. Stefan Lotz from Weylchem (Germany) and Peter Cairns from Arran Chemical Company (Ireland) for their valuable information and the supply of additional samples.

We also thank our colleagues for performing all the NMR, IR, MS, and HPLC-MS analytical tests, in particular, Francis Roll, Eva Buergin, Christian Guenat, Fabrice Houdiere, Elodie Letot, Ingo Muckenschnabel, Thomas Lochmann, Lukas Oberer, Susanne Osswald, and Michael Ritzau.

■ ABBREVIATIONS

BP = boiling point
CDI = carbonyl diimidazole
COPD = chronic obstructive pulmunary disease
CPMA = cyclopentyl mandelic acid
DMF = *N*,*N*-dimethylformamide
DS = drug substance
ET = electron transfer
GLC = gas−liquid chromatography
h = hour
HSE = Health, Safety, and Environment
nd = not determined
PAT = Process Analytical Technologies
oos = out of specification
QbD = Quality by Design
QRA = Quality Risk Analyses
Rt = retention time
RRt = relative retention time
TBME = *tert*-butylmethyl ether
TFA = trifluoroacetic acid
THF = tetrahydrofuran
rt = room temperature

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(14) Citric acid, hydrochloric acid, and sulfuric acid were used.
(15) The aqueous phase after extraction of the product contains beside all the DMF some **3** and is therefore incinerated.
(16) See ref 6c.
(17) In the literature, the diastereomers are often referred to as erythro and threo. As the reconstruction of this denotation is not obvious and sometimes not consistent, we describe the diastereomers as SR/RS and RR/SS, respectively. Paasivitra, J.; Pentilä, A.; Andersen, L. *Finn. Chem. Lett.* **1975**, 94.
(18) These steps can be run in a one-pot fashion. Only because of a final filtration prior to the crystallization, a second reactor is needed into which the warm solution of the crude product **5** is filtered. Even the recrystallization is performed without isolating the wet filter cake. It is redissolved on the nutsch, and the solution is transferred back into the crystallizer.
(19) Possible purifications (e.g., extraction by salt formation as mentioned but of course also chromatographic means) would turn the synthetic process into a multipot process which we want to overcome.
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(29) 1,2,4-Tribromobutane **9a** was detected in ample amounts in commercially available samples of 1,4-dibromo-butan-2-ol **9**.
(30) For example, in a 6% solution of **2** in acetonitrile an initially spiked concentration of ethyl chloroacetate **17c** (2.8% rel. to 2) is declining to 0.01% within 21 h. Compounds **9**, **9a**, **10**, **16b**, and **17b** react vigorously when mixed with neat **2**.
(31) Some of the quaternary ammonium salts are known compounds: **28**: Lunsford, C. D. (A. H. Robins Co., Inc.) GB 1170831 19691119. **29**: Mandava, N.; Fodor, G. *J. Lieb. Ann. Chem.* **1970**, *741*, 167.
(32) The substitution−elimination mode of tribromobutane **9a** in its reaction with bases to form bromoalkenes like **25** is known. See: Lespieau, P. *Compt. Rend.* **1908**, *146*, 1035.
(33) (a) 3-Hydroxy-tetrahydrofuran **31** was detected in batches of **2**. (b) **9a**, **30**, and **31** are commercially available. (c) The preparation of **30** from **9a** is described by: Hojo, K. et al. in EP 0218 249 filed 08.10.1986. (d) The commercially available **9**, contaminated with **9a**, forms a mixture of **2** and **30** when reacted with methylamine.
(34) Astonishingly, **32** was not yet reported in the literature as a byproduct of this reaction.
(35) Its formation is explained by the base-induced transformation of 1,4-dibromo-2-butanol **9** into 4-bromo-1,2-epoxybutane which can be attacked by methylamine at C-1 (forming **2**) or C-2 forming **33**; the NaOH induced epoxide ring formation of the analogous 1,4-dichloro-2-butanol is described in: Johnson, J. Y. Johnson, G. W. GB 692755, June 10th, 1953. Reppe.; et al. *J. Lieb. Ann. Chem.* **1955**, *596*, 80−158 (in particular page 142).
(36) (a) Preparatively aminoxides are obtained with oxygen (air) under pressure at 100°C; hydroxen peroxide and per-carboxylic acids react more smoothly, and organoperoxide requires transition metal catalysts. (b) Doser, K.; Hemmer, R. In *Houben Wyl − Methoden der Organischen Chemie*, Bd. E16a, G. Thieme Verlag Stuttgart: New York, 1990; p 404ff.
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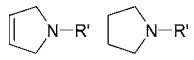
(39) Considering route C in Scheme 3, incomplete hydroboration−oxidation in the last step to **13** would leave 2,5-dihydro-1*H*-pyrroles R_2N-R' [R_2 = $(-CH_2-\mathbf{CH=CH}-CH_2-)$] with R′ = CH_2Ph, H, and Me in **13**, **14**, and **2**, respectively. Hydrogenolytic conditions applied for the conversion of **13** to **14** and **2** would lead to the corresponding saturated pyrrolidines R_2N-R' [R_2= $(-CH_2-\mathbf{CH_2-CH_2}-CH_2-)$]. Except the *N*-benzyl derivates, all compounds have low boiling points (~80−90 °C/760 Torr) which will separate them during the purification of **2** by distillation, and separation by GC from **2** was proven.



(40) Although it was shown that the alkylating agents (**8**, **9**, **9a**, **10**, **15**, **16b**/**c**, **17b**/**c**) could not be present in **2**, they were tested by the GC method applied for the analyses of **2** which reveals their separation from compound **2**.
(41) LOQ (limit of quantification) is 0.05%; LOD (limit of detection) would be even smaller.
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(50) A remark may be allowed: in view of the prior art already published years ago, it is often not reasonable that essentially identical processes can be patented again years later.
(51) A: 28%, B: 38%, C: 50% (86% ester), D: 53%.
(52) If they know at all, vendors and manufacturers do not readily disclose their details, e.g., origin and structure of byproducts.
(53) The ranges for the content of product and byproduct appear to be quite large; they are based on five experiments using different experimental conditions varying the Grignard reagent (cyclopentyl-magnesiumbromide and -chloride), the solvent (diethylether and THF, as well as combinations with toluene), and the starting material (methyl phenylglyoxylate **36a** and the acid **36**). The optimization was not anticipated.
(54) The Grignard reaction, however, is usually complete, and no ester **36n** is left.
(55) Although its preparation needs drastic conditions, see vide infra.
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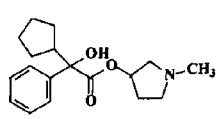
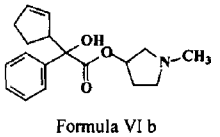
(61) We ruled out two other possibilities: (a) cyclopentene, formed from cyclopentyl-magnesium halide by the β-hydride-transfer, forms a cyclopenten-3-yl-magnesium halide by proton transfer, which adds to **36n**, or (b) cylopentene reacts as an olefin in an Lewis acid catalyzed ene-reaction with **36a** to form **40a**. Simply adding cyclopentene in Grignard-reactions of cyclopentyl-magnesium halide does *not* alter the content of the unsaturated compounds **40** or **41**.

(62) Kaushik, V. K.; Khan, M. U.; Sivakumaran, M. (Aurobindo Pharma Ltd. India), IN2006CH02236 A 20081128. As the patent is not easily available, the mentioned structures of desmethyl-glycopyrrolate VIa (= **3**) and "alkene desmethyl glycopyrrolate VIb" are shown as they are depicted in the patent.



Formula VI a



Formula VI b

(63) We screened the vendors named in SciFinder and have ordered several samples (see Supporting Information).

(64) **45** is mentioned in two patents: (a) Sahyun, M.; Faust, J. A. (Sahyun Laboratories) US 19581001. (b) Moriya, M.; Sakamoto, T.; Ishikawa, M.; Kanatani, A.; Fukami, T. (Banyu Pharmaceutical Co., Ltd., Japan) WO 2004069798 A1 20040819.

(65) Partly transesterification in A forms sodium ethanolate which reacts instead of sodium cyclopentanolate to give rise to substantial amounts of the ethyloxylated compound **46** besides the desired **38**. **46** is a known compound prepared by alkylation of mandelic acid with diethylsulfate. See: Reeve, W.; Pickert, P. E. *J. Am. Chem. Soc.* **1957**, *79*, 1932.

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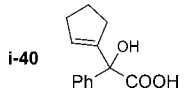
(67) Ikemoto, T. (Sumika Fine Chemicals) EP 1 205 464 A2, 15.05.2002.

(68) Such mixtures are more easily accessible and might still be valuable as reference samples.

(69) A third possible isomere (**i-40**) was prepared following a literature reference: Grover, P. T.; Bhongle, N. N.; Wald, S. A.; Senanayake, C. H. *J. Org. Chem.* **2000**, *65*, 6283. See:



(70) (a) Scholz, V. K.; Spillmann, M.; Tagmann, E.; Hoffmann, K. *Helv. Chim. Acta* **1952**, *35*, 2016. (b) Blicke, F. F.; Raffelson, H. *J. Am. Chem. Soc.* **1952**, *74*, 1730. (c) Bailey, W. F.; Ovasaka, T. V. *J. Am. Chem. Soc.* **1993**, *115*, 3080.

(71) (a) *p*-Chlorophenyl-glyoxylic acid **47** is described: Nimitz, J. S.; Mosher, H. S. *J. Org. Chem.* **1981**, *46*, 211. (b) The reaction of cyclopentyl-magnesium bromide with *p*-chlorobenzoylformic acid **47** is described to yield 12% of **45**: Sahyun, M.; Faust, J. A. US 3009915 19611121. For the reaction with cyclohexylmagnesium bromide see BE 628.563, 17.06.1963.

(72) For one of the isomeres of **57** (not separated from the drug substance), see the discussion vide infra.

(73) This would otherwise not be granted as the impurities might be buried under the peak of the DS,

(74) A new supplier for B1 was used (see Table 2, supplier "a"). Recrystallization of drug substance was performed twice.

(75) The limit of detection was determined to be 0.015%; <0.015% indicates not detected; two figures indicate the number for the isomeres of the corresponding compound.

(76) c^0: content of the impuritiy before crystallization (area % in HPLC). c^K: content of the impuritiy in the recrystallized material. c^{ML}: content of the impurity in the mother liquor. y: yield of the crystallization ($0 < y < 1$), $y = m^K/M$. m^K: amount of crystallized material. M: amount of material before crystallization. For derivation of this equation and data, see the Supporting Information.

(77) Remember the crude drug substance **5** is obtained by crystallization.

(78) Job, A.; Baskarov, D.; Krahwinkel, R.; Hieronymi, A. EP 2 112 137 A1, 28. Okt. 2009.

(79) Allmendinger, T., Bixel, D., unpublished results

(80) Even the reaction with water is slow, and **7** can be detected in HPLC and HPLC-MS after passing the HPLC column in an aqueous eluting system.

(81) **1**, DMF, CDI, rt, 0.5 h; +**2**, 60 °C, 18 h.

(82) It is therefore astonishing that published and patented processes use methanol as cosolvent for the recrystallization; see reference 6c. It explains however the mentioning of **1a** in the Pharmacopoeia as an impurity in gylcopyrrolate, not as a remedy to its intermediacy in the original synthesis but as a degradation product formed during DS-recrystallization using methanol.

(83) More hindered alcohols like 2-propanol, likely to be even less reactive than 1-propanol towards the transesterification of **6**, were examined; however, the solubility of the drug substance turned out to be too low requiring much solvent and large vessels for manufacturing.

(84) Compared to **6**, **1c** is very nonpolar and does not elute in the DS HPLC release method. This can however be easily checked using the HPLC method for the starting material **1**, which separates **6** and **1c** easily.

(85) In fact, two of our vendors were willing to provide such information and samples; they are acknowledged.

(86) There is a patent for the preparation of methyl phenylglyoxylate by the oxidation of methyl mandelate using elemental chlorine mentioning chlorinated byproducts, however, mostly chlorinated at the O–CH_3 functionality: Eberhard, P.; Hickmann, E. (BASF A.-G., Fed. Rep. Ger.) DE 2734207 19790208.

(87) (a) **70** - Radwan, A. S.; Negm, S.; Melek, F. R. *Egypt J. Chem.* **1980**, *23*, 265. (b) **71**: Putochin, N. J. *Ber. Dt. Chem. Ges.* **1922**, *55*, 2749. (c) **72**, $n = 2$ equals prolinol. (d) **72**, $n = 3$: Roussi, G.; Zhang, J. *Tetrahedron* **1991**, *47*, 5161. (e) **73**, $n = m = 2$ (dehydrating): Pugin, B.; Venanzi, L. M. *J. Am. Chem. Soc.* **1983**, *105*, 6877–81. (f) **73**, $n = 2$, $m = 3$; $n = 3$, $m = 4$: Aebi, J.; Binggeli, A.; Green, L.; Hartmann, G.; Maerki, H. P.; Mattei, P.; Ricklin, F.; Roche, O. WO 2009013211 A2 20090129. (g) **73**, $n = 3$, $m = 2$: Santaniello, E.; Manzocchi, A.; Biondi, P. A.; Simonic, T. *Experientia* **1982**, *38*, 782. (h) **73**, $n = m = 3$: Petersen, U.; Schenke, T.; Grohe, K.; Schriewer, M.; Haller, I.; Metzger, K. G.; Endermann, R.; Zeiler, H. J., EP 326916 A2 19890809. (i) **73**, $n = 3$, $m = 4$: Chu, D. T.; Cooper, C. S., WO 9210191 A1 19920625, p 52.

(88) It would anyway be a potential impurity of **2** obtained by method B (see Scheme 3).

(89) (a) ACD/Spec Manager, www.acdlabs.com (accessed Oct 18, 2012). (b) Blinov, K. A.; Smurnyy, Y. D.; Elyashberg, M. E.; Churanova, T. S.; Kvasha, M.; Steinbeck, C.; Lefebvre, B. A.; Williams, A. J. *J. Chem. Inf. Model.* **2008**, *48*, 550–555. (c) Blinov, K. A.; Smurnyy, Y. D.; Churanova, T. S.; Elyashberg, M. E.; Williams, A. J. *Chemom. Intell. Lab. Syst.* **2009**, *97*, 91–97.

(90) Himmelsbach, F.; Langkopf, E.; Blech, S.; Jung, B.; Baum, E.; Solca, F., WO 2002050043 A1.

(91) In such cases, orders were placed to save time and effort. However, in only a few cases the samples were delivered immediately; in other cases, samples needed to be prepared which took some time or were just not available. It seams like some companies have matched the CAS-number listing with their portfolio and claim to get a hold on everything which is not always the case. Sometimes it is better to just make a compound by oneself, like, e.g., **38**, which was offered for a huge price and delivered in questionable quality. With some advice, a trainee was able to prepare it much better.

(92) Not here, but for similar studies in other projects, byproducts prepared on reasonable scale did not undergo the proposed conversion according the conditions of the next step. As an impurity "diluted" in the actual starting material of a particular step, they undergo the corresponding transformation.

(93) Excluding those whose physical properties make their presence in **2** very unlikely (much lower or higher boiling point).

(94) **1c** is too nonpolar to elute during the HPLC analyses, and **69** has a too small UV-factor.

dx.doi.org/10.1021/op3001788 | *Org. Process Res. Dev.* 2012, 16, 1754−1769