Carry Over of Impurities - a detailed exemplification for glycopyrrolate (NVA237)

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Supporting information

Experimental section

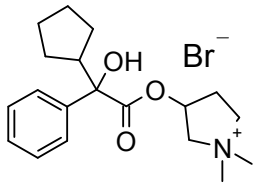
General Methods. Reactions were carried out using dry glassware under an atmosphere of dry nitrogen and magnetically or mechanically stirred, unless noted otherwise. Reagents were purchased from commercial suppliers (Acros, Aldrich, Fluka, Lancaster) and used without further purification, unless noted otherwise. Analytical grade solvents were used as received for reactions, for extractions and chromatographic purifications, unless otherwise stated. Deuterated solvents were obtained from Armar Chemicals, Switzerland. Thin Layer Chromatography (TLC) were used for monitoring reactions and carried out using Merck silica gel 60 F_{254} plates and visualized with UV light, except as indicated otherwise. Flash Chromatography (FC) was performed using Merck silica gel 60 (230-400 Mesh) at a pressure of *ca.* 0.3 bar.[1] Eluents and R_f are indicated. Concentration under reduced pressure was performed at 40°C. Yields refer to chromatographically purified or recrystallized, spectroscopically pure compounds. Melting points were measured on a Büchi SMP-20 apparatus in open glass capillaries and are uncorrected. ^{1}H-NMR spectra were recorded on Bruker DPX 300 MHz or on Bruker DMX 500 MHz spectrometer at 298K in the indicated deuterated solvent, unless otherwise stated. Data are reported as follow: chemical shift (δ, ppm), integration, multiplicity (s, singulet; d, doublet; t, triplet; q, quartet; m, multiplet or not resolved signal; br, broad signal), coupling constant(s) (*J*, Hz). All signals were referenced to the internal solvent signal as standard ($CDCl_3$, δ 7.26; CD_3OD, δ 3.31; DMSO, δ 2.50). ^{13}C-NMR spectra were recorded with ^{1}H-decoupling on Bruker DPX 75 MHz or on Bruker DMX 125 MHz spectrometer at 298K in the indicated deuterated solvent, unless otherwise stated. All signals were referenced to the internal solvent signal as standard ($CDCl_3$, δ 77.0; CD_3OD, δ 49.0; DMSO, δ 39.5). Mass spectra were obtained from the Mass Spectroscopy Service from Novartis Pharma AG, Basel (Switzerland). Elemental analyses were obtained from the Analytical Service from Solvias AG, Basel (Switzerland).

GC-analyses for **2** were performed using gas chromatographs with FID detection (300°C), columns with 100% polysiloxan using hydrogen as carrier gas, temperature gradients 80-290°C.

HPLC-analyses of **1** were performed using YMC ODS-AQ columns with gradients of acetonitrile and aqueous phosphate buffers at pH 2.5 or XTerra RP18 3.5um using gradients of aqu. phosphoric acid and acetonitrile.

Samples were purchased from Aldrich (**9**, **10**, **12**, **13**, **14**, **18**, **19**, **35b**, **35c**), TCI Europe Fine Chemicals (**9**, **30**), UkrOrg Synthesis Building Blocks (**8**), J&W Pharmlab (**33**). Samples of CPMA (**1**) are purchased from (in alphabetical order not matching numeration a-k in table 2) ABCR, Acros, Alkalimetals, Allichem LLC, APAC, Atlantic SciTech Group, Arran, JWF, Shanghai FWD Chemicals, Shanghai Yiyi Maoyi Co., Synergetica-Changzhou, Wako.

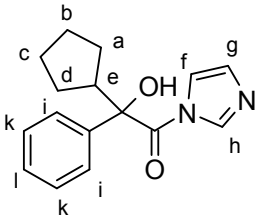
3-(2-Cyclopentyl-2-hydroxy-2-phenyl-acetoxy)-1,1-dimethyl-pyrrolidinium bromide (glycopyrrolate) **6** (new, shortened, one pot process). Attention: Compound **3** is a compound causing reversible exogeneous psychosis; preparation and handling needs to be performed with extreme care.



30 g (0.136mol) of cyclopentyl mandelic acid **1**, dissolved in 135 g dimethylformamide (DMF), was treated with 27 g carbonyl diimidazole at 18°C (in portions) to form the "active amide". After the addition of 16.9 g of 1-methyl-pyrrolidin-3-ol, the mixture was heated to 60°C within 1 hour and stirred for 18 hours at this temperature. The mixture was cooled and 200 g water was added. The mixture was extracted with 200 g toluene and the extract was washed with water three times. The organic phase was concentrated to obtain a 50% solution of cyclopentyl- hydroxy-phenyl-acetic acid 1-methyl-pyrrolidin-3-yl ester **3** in toluene. This solution was diluted with 120 g of n-propanol and cooled to 0°C. 16.8 g methyl bromide was introduced and the mixture was stirred for 2 hours and then gradually heated to 60°C. The product crystallized on cooling and was isolated by filtration to obtain 22.7 g (0.057mol) **5** after drying. The solid obtained was further recrystallised from n-propanol (1:10 wt) once or twice to

give pure (3S,2'R)- and (3R,2'S)-3-[(cyclopentyl-hydroxyphenylacetyl)-oxy]-1,1-dimethylpyrrolidinium bromide i.e. purity > 99.9% as determined by high performance liquid chromatography (HPLC).

2-Cyclopentyl-2-hydroxy-1-imidazol-1-yl-2-phenyl-ethanone (**7**)



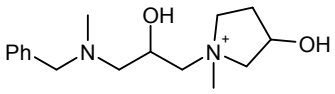
Cycloptenyl mandelic acid (10g), suspended in 30ml of acetonitrile, was treated at 0°C with carbonyldiimidazole (10.3g). The mixture was warmed to room temperature for 2h whereby carbon dioxide evolved and a solid was formed. The mixture was cooled to 5°C and filtered. The solid was washed with acetonitrile and dried in vacuo to obtain **7** (7.3g). High resolution MS-spectroscopy revealed the molecular formula of the compound (as M+H) to be $C_{16}H_{19}O_2N_2$ with an exact mass of 271.14414 (0.14575ppm deviation from the calculated value).

^{1}H-NMR-spectroscopy (600MHz, DMSO-d_6): δ = 1.03-1.07 (m, 1H), 1.25-1.30 (m, 1H), 1.35-1.40 (m, 1H), 1.40-1.50 (m, 1H), 1.53-1.56 (m, 2H), 1-60-1.67 (m, 1H), 1.75-1.84 (m, 1H), [1.03 – 1.85, 8H, **a-d**); 2.7-2.9 (m, 1H, **e**); 3.40 (br, 1H, OH); 6.79 (1H, **g**); 6.91 (1H, **f**); 7.29 (1H, **l**); 7.39 (2H, **k**); 7.49 (2H, **i**); 7.65 (1H, **h**). IR-spectroscopy (measured as a solid film on a Bruker Tensor 27 FTIR spectrometer over a wave number range of 4000-600 cm^{-1} with a resolution of 4 cm^{-1}): 3300 ~ 2500 (O-H stretching), 3167, 3151, 3120 (Imidazole CH stretching), 2956, 2868 (Cyclopentyl CH stretching), 1727 (C=O stretching), 1600, 1538, 1469 (Aromatic rings stretching), 735 (mono-subst. benzene CH o.o.p. bending), 704 (Mono-subst. benzene ring o.o.p. bending).

Quarternisation of 1-methyl pyrrolidin-3-ol 2 with alkylating agents (see scheme 5). In individual 10ml screw cap vials, small amounts (0.19g each) of the commercially available compounds **8** (benzyl-methyl-oxiranylmethyl-amine), **9** (1,4-dibromobutan-2-ol), **9a** (1,2,4-tribromobutane, a by-product in the commercial sample of **9**), **10** (1,4-dichloro-2Z-butene),[2] **15** (ethyl acrylate), **16b/c** (benzylbromide and benzylchloride), and **17b/c** (ethyl bromo- and chloroacetate) were added to each 2ml (1.97g) of 1-methyl pyrrolidin-3-ol. The reactions of **10** was very exothermic and the reactions of **16b** and **17b** were

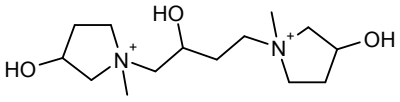
moderately exothermic. The vials were closed and kept at room temperature for 1-3 days and subjected to LC-MS- analyses to detect in each case excess **2** (MH^+ = 102) and the quarternary ammoniumsalts (see scheme 5).

1-[3-(Benzyl-methyl-amino)-2-hydroxy-propyl]-3-hydroxy-1-methyl-pyrrolidinium bromide (**23**). The LC-MS-analyses showed the masses 279



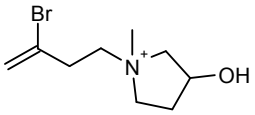
in the ES+ mode and 369 in the ES- mode, revealing the molecular formulas of $C_{16}H_{27}N_2O_2$ (M^+) and $C_{16}H_{27}N_2O_2 + 2xCHO_2$ ($M^+ + 2x\ HCOO^-$).

1,1'-(2-Hydroxy-butane-1,4-diyl)bis[1-methyl-3-hydroxy]pyrrolidinium dibromide (**24**) The compound crystallized



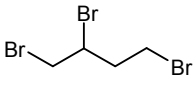
from the mixture of **9** (and **9a)** and excess **2**. The hygroscopic solid was filtered and washed with **2**. The LC-MS shows the m/z-values 137, 273 and 353/355 (M^{2+}, M^{2+}-H, M^{2+}+Br^-) matching with $C_{14}H_{30}N_2O_3$. Using 1,4-dichlorobutan-2-ol, crystalline 1,1'-(2-hydroxy-butane-1,4-diyl)bis[1-methyl-3-hydroxy]-pyrrolidinium dichloride was formed as a hygroscopic compound, verified by HR-MS: 137.11230 (M^{2+}; +0.2ppm from the value calculated for $C_{14}H_{30}N_2O_3^{2+}$) and 273.21711 [$(M-H)^+$, -0.6ppm from the value calculated for $C_{14}H_{29}N_2O_3^+$) and 319.22250 [$(M+HCOO)^+$, -0.8ppm from the value calculated for $C_{15}H_{31}N_2O_5^+$).

1-(3-bromo-but-3-enyl)-3-hydroxy-1-methyl-pyrrolidinium bromide (**25**). The compound was present in the mother liquor of the above mentioned preparation



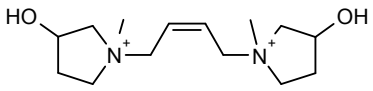
stemming from **9a**, the impurity in the sample of **9** used.[3] The LC-MS reveals the structure of **25** $C_9H_{17}BrNO$ by its m/z = 234/236.

1,2,4-Tribromobutane **9a** was easily prepared by a slight modification of a published



procedure:[4] bromine (11.3g, 0.071mol, careful) was added slowly to a solution of 4-bromo-1-butene (9.6g, 0.071mol) in dichloromethane (45g) at -5 - +5°C. After dilution with dichloromethane (40mL), the mixture was washed with aq. sodium hydrogensulfite and water and the organic phase was evaporated to obtain **9a** (18.08g, 86%) as a colorless oil. ^{13}C-NMR (100MHz, $CDCl_3$): δ = 30.3 (C-4), 35.7 (C-1), 38.9 (C-3), 50.1 (C-2). These ^{13}C-NMR-data are in full agreement with the data of the minor component **9a** in the sample of **9**.

(Z)-1,1'-(but-2-ene-1,4-diyl)bis(3-hydroxy-1-methylpyrrolidinium) chloride (**26**): To a solution of 5.00g (49.4mmol) **2** in 14.3g acetonitrile

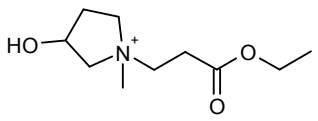


was added at once 1.13g of (Z)-1,4-dichlorobut-2-ene (**10**, careful, very toxic). The stirred mixture became turbid after 5 minutes and a precipitate was formed after 15 minutes. The solid was isolated by fitration, washed with acetonitrile and dried in vacuo at 40°C to get 2.61g (88%) of **26**.

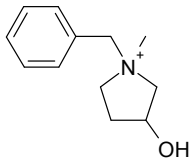
HR-MS: Calculated for M^{2+} $C_{14}H_{28}O_2N_2$ (256.2151), found 256.21412 (m/z = 128.10706), deviation 0.52746ppm; calculated for M^{2+} + Cl^- $C_{14}H_{28}O_2N_2Cl$ (291.18402), found 291.18317, deviation -0.72032ppm.

^{1}H-NMR (600 MHz, DMSO-d_6): δ = 2.03 (br., 2H), 2.44 – 2.54 (m, 2H), 3.14, 3.26, 3.49 (s, br., 6H), 3.5 – 3.9 (m, 8H), 4.49 – 4.4.63 (m, 6H), 6.04 – 6.08 (m, 2H, OH), 6.25 – 6.33 (m, 2H, -CH=CH-).

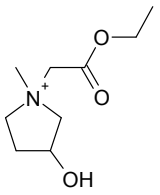
1-(2-Ethoxycarbonyl-ethyl)-3-hydroxy-1-methyl-pyrrolidinium (**27**) was formed in a mixture of **2** and ethyl acrylate **15**. Its structure was comfirmed by HR-MS. The exact mass, calculated for $C_{10}H_{20}NO_3$ is 202.14377, found 202.14369 (-0.4ppm).



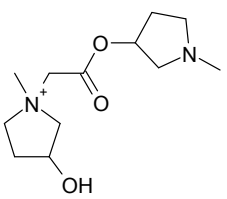
1-Benzyl-3-hydroxy-1-methyl-pyrrolidinium halides (**28**) were formed in mixtures of **2** and benzyl chloride **16c** or benzylbromide (**16b**, careful, exothermic reaction) as confirmed by HPLC-MS; the spectra showed the masses 192 (for M^+, $C_{12}H_{18}NO$), 383 (for $2M^+$-H, $C_{24}H_{35}N_2O_2$) and 429 (for $2xM^+ + HCOO^-$).



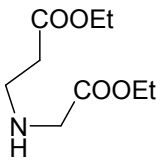
1-Ethoxycarbonylmethyl-3-hydroxy-1-methyl-pyrrolidinium bromide and **chloride** (**29a**). The compound was described and prepared by refluxing a mixture of ethyl bromoacetate and **2** in acetonitrile.[5] Refluxing was not necessary, ethyl chloro- and bromoacetate both reacted with **2** in acetonitrile at room temperature in less than 5 days to form **29a**. MS-analyses revealed the masses of M^+ ($C_9H_{18}NO_3$, 188), $2M^+ - H^+$ (375), $2M^+ + HCOO^-$ (421), $2M^+ + Br^-$ (455/457) and in the ES- mode $M^+ + 2Br^-$ (346/348/350). ^{1}H-NMR (400 MHz, DMSO-d_6): δ =1.28 (t, 7.15Hz, 3H), 2.15 – 2.3 (m, 1H), 2.5 – 2.65 (m, 1H), 3.32 and 3.43 (s, 2:1, 3H), 3.8 – 3.9 (m, 3H), 3.9 – 4.1 (m, 1H), 4.29 (q, 7.15Hz, 2H), 4.39 and 4.38 (s, 1:2, 2H), 4.70 (s, 1H), 4.75 (br. s, 1H).

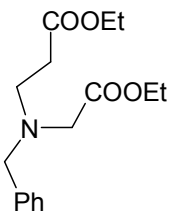


Reacting ethyl bromoacetate in neat excess **2** at room temperature, product **29a** was further transformed to **29b**, which turned out to be the only product when *methyl* bromoacetate was used. The ethyl (or methyl) ester in **29a -** activated by the adjacent positive charge - was transesterified with excess **2** to form 3-hydroxy-1-methyl-1-(1-methyl-pyrrolidin-3-yloxycarbonyl-methyl)-pyrrolidinium bromide **29b** which was justified by the mass of 243 for $C_{12}H_{23}N_2O_3$.



3-(Ethoxycarbonylmethyl-amino)-propionic acid ethyl ester (**20**) and **3-(benzyl-ethoxy-carbonylmethyl-amino)-propionic acid ethyl ester** (**21**)

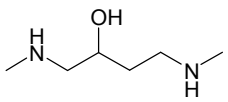




Following the procedure of Roglans,[6] 20g (0.143mol) of Glycin ethylester hydrochloride was added to a solution of 5.67g (0.142mol) of sodium hydroxide in 60g water at 0°C and the mixture was stirred for 10 minutes. After the addition of 14.3g (0.143mol) ethyl acrylate

the mixture was slowly warmed to room temperature, stirred over night and was then extracted with dichloromethane 3 times. The combined extracts were dried over sodium sulfate, filtered and evaporated to obtain 17.8g of **20** as slightly yellow oil. ^{1}H-NMR (600MHz, DMOS-d_6) 1.17-1.20 (two t, 6H), 2.4 (m, 2H), 2.75 (m, 2H), 3.3 (s, 2H), 4.04 and 4.09 (q, 2H each). To a solution of 3g (14.8mmol) of crude **20** in 12g of acetonitrile was added 1.4g (16.7mmol) sodium bicarbonate and 2.5g (14.6mmol) of benzylbromide and the mixture was stirred for 18h at room temperature. After the addition of a polyamine containing silica resin[7] (5g) shaking was continued for 2 days. The mixture was filtered and evaporated. The residue was dissolved in dichloromethane and washed with water. The organic phase was evaporated to obtain ethyl 3-(benzyl(2-ethoxy-2-oxoethyl)amino)propanoate (3.1g, 14.7mmol, 72%) as clear, yellow oil. Calculated for $C_{16}H_{24}NO_4$ (M+H): 294.16999, found 294.16986. The compound was identical to a sample prepared by the alkylation of ethyl 3-benzylamino-propionate **18** with ethyl chloroacetate **17c**,[8] or of ethyl N-benzylglycinate with ethyl acrylate;[9] it is also commercially available (Aurora Fine Chemicals).

1,4-Bis-methylamino-butan-2-ol (**32**) was detected as a by-product during the preparation of 1-methyl-pyrrolidin-3-ol (**2**) using method D (see scheme 3). As this compound is not described in the literature, an account for preparation and isolation is given.

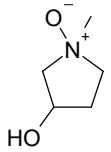


To 160ml of a 20% aqueous solution of methylamine, of 1,4-dibromo-2-butanol (**9**) (22ml, 43g, 0.18mol) was added with stirring and cooling over 4-5 minutes. The mixture was heated within 14h to 100°C and stirred for 2h at that temperature. After cooling to room temperature 60ml of 30% aqu. NaOH were added. Water and excess methylamine were evaporated under reduced pressure to obtain 95g of a residue which was saturated with 6g potassium carbonate and filtered. The filtrate was extracted with 2-butanol (3x20ml) and the extracts were subjected to fractional vacuum distillation to obtain fraction 1 (42g, bp <30°C/25mbar, mainly2-butanol), fraction 2 (5.9g, bp. 38-50°C/5mbar, 2-butanol, **2** and **33**), fraction 3 (7.4g, bp. 60-75°C/1-5mbar). The distillation fractions 3 of four such batches were combined to get 25.8g, of a mixture containing 10% 2-butanol, ~80% **2**, 2% **33** and 3.5%

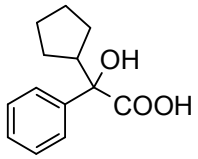
32.[10] This mixture was redistilled using a vigreux-column to afford 13.5g of **2** (98% pure by GC) and 0.9g of a distillation residue consisting of 86% 1,4-bis-dimethylamino-2-butanol (**32**). Having obtained a larger amount of late distillation fractions from a supplier, compound **32** could be distilled under reduced pressure (1mbar) at 92 – 102°C.

^{1}H-NMR (600MHz, DMSO-d_6) 1.39 (m, 1H), 1.50 (m, 1H), 2.25 (s, 3H), 2.27 (s, 3H), 2.37 (m, 2H), 2.54 (m, 2H), 3.61 (m, 1H); ^{1}H-NMR (400MHz, $CDCl_3$) 1.55-1.62 (m, 2H, C-3-H), 2.42 and 2. 44 (s, 3H, each, CH_3N), 2.52-2.62 (m, 2H, C-1H), 2.73-2-81 (m, 1H, C-4-H), 2.88-2.94 (m, 1H, C-4-H), 3.86-3.93 (m, 1H, C-2-H); ^{13}C-NMR (400MHZ, $CDCl_3$) 33.3 (C-3), 36.1 (N-CH_3), 36.3 (N-CH_3), 50.1 (C-4), 58-1 (C-1), 70.4 (C-2). IR (FTIR-microscope in transmission), ν [cm^{-1}]: 3361 (br., strong, N-H, O-H), 2939 (w, C-H), 1636, 1538, 1488, 1387, 1265, 1045, 815. HR-MS, calculated for $C_6H_{17}N_2O$ ($M+H^+$) 133.1341, found 133.13365.

1-Methyl-1-oxy-pyrrolidin-3-ol (**34**): In analogy to a literature reference for a similar compound,[11] a solution of **2** (5.3g, 0.052mol) in ethanol (30mL) was treated with 30% hydrogen peroxide (30mL, 0.26mol) and after stirring for 21h the mixture was diluted with water (200mL) and filtered through a column of Dowex 50 (H^+-form, 50mL); the resin was washed with water (400mL) and the product was eluted with 3% aqueous ammonia (500mL). Water was removed by evaporation at reduced pressure to obtain **34** in quantitative yield as a brownish oily substance consisting of a mixture of 2 isomeres in a ratio of 2:1. ^{13}C-NMR (150MHz, DMSO-d_6), δ = *33.1* and 34,3 (C-4), 55.5 and *56.8* (CH_3), *68.3* and 68.6 (C-5), *68.6* and 70.5 (C-3), 74.1 and *77.1* (C-2). ^{1}H-NMR (600MHz, DMSO-d_6), δ = 1.67, 2.15, 2.3, 2.6 (m, 2H, C-4-H); 3.10, 3.13 (s, 3H, N-CH_3); 3.16-3.66 (m, 4H, C-2,5-H), 3.6 (br, 1H; OH); 4.26, 4.58 (m, 2H, C-3-H). IR (in CH_2Cl_2): ν~ 3400-2800 (OH), 3058, 3044, 2955 (s, C-H), 1463, 1441 (s), 1283, 1202, 1160, 1089 and 1078 (s, C-O), 999, 948 and 923 (N-O), 842, 807(cm^{-1}). ESI-MS 118.1 ($M+H^+$), High resolution MS 118.08636 Δ=0.8ppm $C_5H_{12}NO_2$.



Cylopentyl mandelic acid (**1**), typical procedure for pathway A (*scheme 7*): To a mixture of 20 mL of THF, iodine (2 balls) and magnesium turnings (2.94 g, 121 mmol) was added 2.5g (0.017mol) of bromocyclopentane, after a short time, a strongly exothermic reaction started and the reaction mixture refluxed. The solution of 15.4g (0.104mol) of bromocylcopentane in 50ml of THF was added at such a rate to keep the mixture refluxing. After the end of the addition the mixture was kept at 50°C for 4h and is then cooled to 0°C. A solution of 16.4g (0.1mol) of methyl phenylglyoxylate **36a** in 80mL of THF was then added within 1.5h while the temperature was kept below 10°C. After stirring at r.t. over night, the mixture was poured to ice cold of 1N aq. sulfuric acid (100mL). The layers were separated, the aqueous layer was extracted with toluene twice and the combined organic layers were washed with water and evaporated to obtain 19.6g of crude methyl cyclopentylmandelate (**1a**, MCPM). The crude ester was dissolved in methanol (50ml) and 60ml of 15% aq. sodium hydroxide (0.225mol) were added. After stirring at room temperature over night, the mixture was diluted with water (80ml) and extracted with toluene to remove nonpolar compounds. The aqueous phase containing the sodium salt of **1** was layered with toluene (100ml), warmed to 40°C and acidified by the addition of hydrochloric acid. The layers were separated and the aqueous phase was extracted with warm toluene (100ml) a second time. The combined organic extracts were concentrated by distilling off 140ml of toluene. The mixture was then cooled from 110°C to 0°C in 15h to induce crystallization. The solid was filtered, washed with cold toluene and recrystallized from a mixture of methanol and water (36ml, 28ml respectively) to afford 5.45g (0.025mol, 25%) of pure **1**.



Typical procedure for pathway B (*scheme 7*): The solution of phenylglyoxylic acid **36** (3.75 g, 25 mmol) in THF (30 mL) was cooled to 0 °C and a 25% solution of cyclopentylmagnesium chloride in THF/toluene (38.12 g, 74mmol)[12] was added within about 1 h to keep the temperature below 10 °C. The mixture was then stirred at r.t. overnight and then quenched into aqueous sulfuric acid (50 mL, 1N) and ice. The layers were separated and the aqueous layer was extracted twice with TBME. The combined extracts were washed with water and evaporated to dryness to obtain crude cyclopentylmandelic acid

(4.21 g) which was recrystallized from toluene first and them from methanol/water as above to obtain 0.74g (14%) of pure **1**.

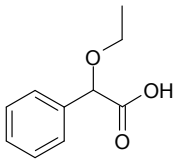
Table 6: composition (area-% by HPLC) of crude **1**, prepared by procedure A (from **36a**) and B (from **36**) and recrystallized from toluene (tol) *and* from methanol/water (m/w).

	1	**36**	**37**	**38***	**39**	**40**	**41**	**	toluene
"A"-crude	70.1	10.0	4.2	2.2	6.5	0.4	0.5	0.3	0.8
"A-tol"	96.8	0.2	1.6	-	0.04	0.02	0.02	-	0.8
"A-mw"	99.8	-	-	-	-	0.02	0.01	-	0.01
"B" Crude	37.4	6.6	10.3	6.2	1.4	5.8	5.2	1.6	1.2
"B-tol"	92.7	0.1	3.1	-	0.04	0.11	0.05	0.07	1.3
"B-mw"	99.2	-	0.01	-	-	0.02	-	-	0.01

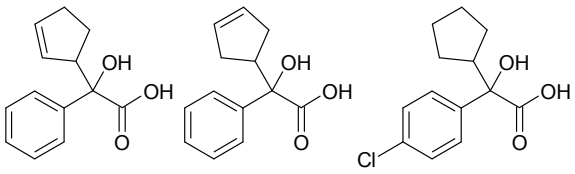
*As **38** is not separating from **1** in the raw material control procedure, it is quantified using another HPLC-method; the data show a strong depletion during the recrysallization from toluene and methanol/water; **the relative retention time of this peak is identical to that of Cl-CPMA **45**, however LC-MS does not support its presence in these samples; furthermore the strong depletion of this compound during recrystallization does not match with the behavior of a sample of **1** really containing **45** (sample of supplier **a** in table 29) which was subjected to recrystallization from toluene or methanol/water; the content of **45** dropped only by a factor of 2 in both cases.

Cyclopentyloxy-phenyl-acetic acid (**38**). The solution of cyclopentanol (14.14g, 0.164mol) in 120ml THF was added within 20 minutes at 10°C to the suspension of sodium hydride (60%, 21.9g, 0.546mol) in 120ml THF followed by the addition of 2-bromo-phenyl-acetic acid (24g, 0.11mol) in 120ml THF. The mixture was warmed to room temperature for 1.5h, then the solvent was evaporated and 400ml water was added with cooling. The aqueous solution was washed with TBME (3*200ml) and acidified with 2N HCl (500ml). The solution was extracted with TBME (3*200ml) and the organic extract was concentrated to get **38** (22.95g, 0.104mol, 95%) as a waxy material. ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1.47 (m, 2H), 1.63 (m, 4H), 1.73 (m, 2H), 4.00 (m, 1H), 4.92 (s, 1H), 7.32 (m, 1H), 7.37 (m, 2H), 7.44 (d, 7Hz, 2H). IR (FTIR-microscope, cm^{-1}): ν = 3400-2600 (br., CH, OH), 1710 (C=O), 1602, 1465, 1455, 1426, 1323, 1272, 1235, 1189, 1177, 1104, 1071, 987, 723, 693. HR-MS: 219.10256 (-0.47ppm from calculated value for $C_{13}H_{15}O_3$ = M-H).

Ethoxy-phenyl-acetic acid (**46**).[13] Similar to **38**, **46** was prepared in 96% yield by using ethanol instead of cyclopentanol. ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1.15 (t, 7.0Hz, 3H), 3.42 (m, 1H), 3.56 (m, 1H), 4.87 (s, 1H), 7.3-7.45 (m, 5H), 12.79 (br.s., 1H). (FTIR-microscope, cm^{-1}): ν = 3600-2600 (br. OH, CH), 1726 (C=O), 1603, 1495, 1455, 1408, 1218, 1185, 1111, 1074, 1033, 723, 698. HR-MS: 179.07127 (-0.53 ppm from calculated value for $C_{10}H_{11}O_3$ = M-H).

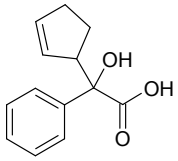


Cyclopent-2-enyl-hydroxy-phenyl-acetic acid (**40**) and **cyclopent-3-enyl-hydroxy-phenyl-acetic acid** (**41**) by chromatographic separation from **1**. An impure commercial sample of CPMA (**1**, 2g) was chromatographically separated in 4 runs using a column which was packed with 300g YMC ODS (RP18) as slurry in isopropanol. After conditioning, the elution with a flow rate of 100ml/min was done with a gradient of water/acetonitrile (both with 0.1and 0.05% TFA). The by-products **40**/**41** and **45** are eluting before and after the main compound (**1**) respectively.



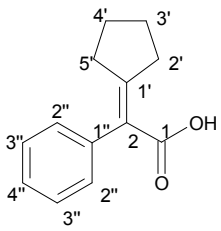
Cyclopent-2-enyl-hydroxy-phenyl-acetic acid (**40**) by synthesis.[14]

0.21g (1.58mmol) ethyl phenylglyoxylate dissolved in 2ml of dichloromethane was cooled to -10°C and treated with 0.59g (2.1mmol) of cyclopent-2enyl-trimethylsilane and 1.6ml of a 5M solution of titanium tetrachloride in dichloromethane. After stirring for 1h at that temperature the reaction mixture was quenched with water and extracted with dichloromethane. The organic phase was dried over sodium sulfate, evaporated and chromatographed on silica to get cyclopent-2-enyl-hydroxy-phenyl-acetic acid ethyl ester (**40b**) as a mixture of two isomers contaminated with ethyl mandelate and ethyl benzoate [^{1}H-NMR ($CDCl_3$, 400MHz), δ = 1.3 (m, O-C-CH_3 mi), 1.6-1.8 (m), 2.0 (m), 2.2-2.6 (m), 3.8 (m), 4.2 and 4.25 (qu., O-CH_3-Me), 5.2 and 5.8 (m, CH=CH, mji), 5.5 and 6.0 (m, CH=CH, mni), 7.28 (m), 7.35 (t), 7.52 (t), 7.66 (m), 8.02 (d).



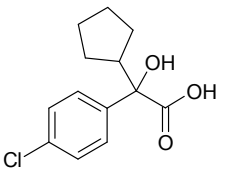
The compound was dissolved in 5ml ethanol and 0.14g NaOH was added. The mixture was stirred for two hours. After quenching with water, the mixture was extracted with TBME. The aqueous phase was acidified with 2N HCl and extracted with TBME. The extract was evaporated to get 0.14g of the desired acid as a mixture of two isomers. The crude product contains phenylglyoxylic as well as benzoic acid. $C_{13}H_{14}O_3$, calculated mass for M-H: 217.08702, found: 217.08710 (0.4ppm); 1H-NMR (400MHz, $CDCl_3$): 1.6 (m), 1.73 (m), 1.85 (m), 2.1 (m), 2.2-2.55 (m), (1.6-2.6, 4H), 3.83 (br., 1H), 5.2 (m), 5.6 (m), 5.83 (m), 6.08 (m), (5.2 – 6.1, 2H); 7.3-7.7 (m, 5H).

Cyclopentylidene-phenyl-acetic acid (**42**). A mixture of cyclopentyl mandelic acid (**1**) (111.5g , 0.506mol), water (230g) and 37% hydrochloric acid (214g, 2.172mol) was refluxed for 49h. During that time an almost clear solution was formed. The mixture was cooled to 20°C within 2h to obtain a thick suspension which was filtered.



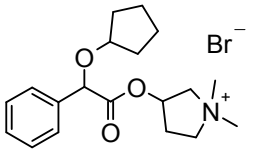
The solid was washed with water (2L) and dried in vacuum to obtain 93.5g (0.462mol, 91.4%) of the crude product. Recrystallization from 100g hot ethanol furnished 64g (63%) of pure **42**. ^{1}H-NMR (400 MHz, $CDCl_3$): δ = 1.63 (dq, 7.03Hz, 6.86Hz, 2H); 1.81 (q, 6.96Hz, 2H); 2.26 (t, 7.15Hz, 2H); 2.95 (t, 7.15Hz, 2H); 7.22 (d, 6.78Hz, 2H); 7.32 (m, 1H); 7.40 (t, 7.28Hz, 2H); 11.8 (br. s., 1H). ^{1}H-NMR (600 MHz, DMSO-d_6): 1.52 (C-4'-H), 1.69 (C-3'-H), 2.13 (C-5'-H), 2.79 (C-2'-H), 7.15 (C-2''-H), 7,23 (C-4''-H), 7.32 (C-3''-H), 12,13 (OH). ^{13}C-NMR (100 MHz, $CDCl_3$): δ = 25.75, 26.85 (C-3', C-4'); 34.63, 36.11 (C-2', C-5'); 125.11 (C-2); 127.07 (C-4''); 128.25 (C-2''); 129.38 (C-3'); 138.75 (C-1''); 168.14 (C-1'); 172.88 (C-1). ^{13}C-NMR (150 MHz, DMSO-d_6): 25.40 (C-3'), 26.26 (C-4'), 33.61 (C-2'), 34.62 (C-5'), 126.18 (C-2), 126.46 (C-4''), 127,90 (C-3''), 129.02 (C-2''), 139.31 (C-1''), 160.62 (C-1'), 168.21 (C-1). IR (FT-IR-microscope): ν [cm^{-1}] 3100 – 2500 (O-H), 3059 (arom. C-H), 2958 (aliph. C-H), 1678 (C=O), 1614, 1597 (arom. C-C), 1496 (arom), 1414, 1286, 1275, 1251 (C-O, acid), 726, 699.

(4-Chloro-phenyl)-cyclopentyl-hydroxy-acetic acid (**45**). A solution of 10.5g cyclopentyl-oxo-acetic acid ethyl ester in 400ml of THF was cooled to -78°C and treated with 88ml of a 2M solution of 4-chlorophenyl-magnesiumbromide in diethylether within 30 minutes. The solution was stirred for an additional hour and was then poured onto a mixture of 180ml of a saturated aqueous solution of ammonium chloride and 140ml of ethyl acetate. The phases were separated and the organic phase was washed with water and brine, dried over magnesium sulfate and evaporated to dryness. The residue (20.85g of the crude ester) was diluted with 200ml of THF, 200ml of methanol and 117ml of 1M aqueous LiOH were added and the mixture was heated to reflux for 18 hours. After cooling the volatile solvents were evaporatead and the aqueous residue was extracted with TBME. The aqueous phase was adjusted to pH 1 using 1N HCl and extracted three times with TBME. The extract was washed with water and evaporated to dryness. The residue (11.6g crude product) was dissolved in 17ml of heptanes at 40°C. Upon cooling to 0°C a precipitate was formed which was collected, washed with heptanes and dried in vacuum to get 6.8g of the solid acid.



Elemental analyses - found (calculated): C 61.76 (61.3%); 6.23 (5.94%); Cl 13.55 (13.92%). HR-MS for $C_{13}H_{14}O_3Cl$ 253.06363 (-0.26ppm for M-H). IR (FT-IR microscope in transition; cm^{-1}): 3423 (OH), 3068, 2958, 2870, 1723, 1491, 1453, 1399, 1361, 1320, 1298, 1213, 1181, 1162, 1117, 1096, 1036, 1012, 963, 817, 750, 716, 669. ^{1}H-NMR ($CDCl_3$, 400MHz). δ= 1.2-1.3 (m, 2H); 1.35-1.65 (m, 6H); 2.76 (m) ; 3.45 (s, br, 1H, OH); 7.24 (d, 8.8Hz); 7.53 (d, 8.8Hz); 9.4 (s, br, 1H, COOH). ^{13}C-NMR ($CDCl_3$, 100MHz), δ= 25.85, 26.27, 26.33, 26.89, 47.33, 78.86, 127.47, 128.37, 133.82, 139.41, 180.33.

3-(2-Cyclopentyloxy-2-phenyl-acetoxy)-1,1-dimethyl-pyrrolidinium bromide (**48**). The solution of **38** (10g, 45.5mmol) in DMF (50ml) was treated with CDI (14.7g (91mmol) in portions at room temperature. After leaving the mixture for one hour, **2** (6.89g, 68mmol) the mixture was gradually heated to 50°C and stirred for 24h. After the addition of saturated sodium bicarbonate solution (200ml) the mixture was extracted with TBME (200ml). The extract was washed with water three times and evaporated to dryness. The intermediate

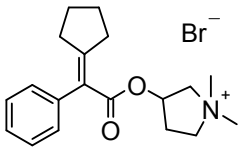


(9.6g, 32mmol) was dissolved in acetone (20ml) and a solution of methylbromide in n-propanol (50g, 23%, 121mmol) was added at once. The mixture was stirred for 3 days and evaporated. The residue was dissolved in water (400ml) , extracted with TBME (2x60ml) and evaporated to get 14.19g of a sticky material.

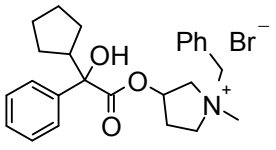
^{1}H-NMR (DMSO-d_6, 400MHz). δ=1.4-1.75 (m, 8H), 2.0-2.2 and 2.6-2.8 (m, 2H), 3.08, 3.16, 3.20, 3.22 (s, 6H, N-CH_3), 3.5-4.0 (m, 4H, $N^+(CH_2)_2$), 4.06 (br., CH-O), 5.05 (s, 1H, Ph-CH(CO)-O), 5.46 (br., 1H, CH-O-CO), 7.2-7.5 (m, 5H); MS: 318; HR-MS: 318.20646 (M^+) 0.3ppm for $C_{19}H_{28}NO_3$, besides some 304.19074 (0.06ppm for $C_{18}H_{26}NO_3$, desmethyl-**48**+H^+); IR (FT-IR microscope in transmission, cm^{-1}): 3419, 2960, 1749, 1633, 1475, 1455, 1322, 1260, 1203, 1174, 1116, 1070, 1018, 990, 736, 700. 13C-NMR (600MHz, MeOD): 22.6, 31.5.

3-Benzoyloxy-1,1-dimethyl-pyrrolidinium bromide (**49**).[15] To the solution of **2** (2.52g, 25mmol) in toluene was added pyridine (1.51g, 19mmol); after cooling to -5°C benzoylchloride (2.06g, 14.7mmol) was added slowly. After stirring for 3 h at rt the mixture was extracted with aqu. sodium bicarbonate and water and evaporated. The residue was dissolved in TBME and extracted with 100ml 1N HCl. The aqu. phase was made alkaline with 2N NaOH and back extracted into TBME. This organic phase was dried and evaporated to get 2.0g of the intermediate. The intermediate was reacted with methylbromide in 1-propanol for 1week. The mixture was evaporated and triturated with TBME to get a solid which was filtered and dried. ^{1}H-NMR (DMSO-d_6, 600MHz). δ= 2.44, 2.82 (br., 2H), 3.31, 3.34 (s, 6H, NCH_3), 3.74, 3.87, 3.97 and 4.10 (br., 2H,), 5.63 (br., CH-O), 7.55 (m, 2H), 7.69 (m, 1H), 8.03 (m, 2H); ^{1}H-NMR (CD_3OD, 600MHz): 2.57 & 2.95 (C-4-H), 3.39 & 3.43 (s, 6H, N-$CH_3)_2$), 3.80 & 3.97 (C-5-H), 4.13 (C-2-H), 5.80 (C-3-H), 7.53 (2H, m-H), 7.67 (1H, p-H), 8.09 (2H, o-H). (MS: 220 (M^+, accounts for $C_{13}H_{18}NO_2$; IR (FT-IR microscope in transmission, cm^{-1}): 3326, 3010, 1710, 1603, 1584, 1574, 1455, 1392, 1317, 1275, 1129, 1973, 1040, 1028, 1005, 964, 928, 859, 711. HR-MS: calculated for $C_{13}H_{18}O_2N$ 220.13321, found 220.13319 (-

0.05ppm). ^{13}C-NMR (600MHz, CD_3OD): 29.72 (C-4), 64.47 (C-5), 69.93 (C-2), 72.35 (C-3), 128.05, 128.67, 129.02, 133.10, 165.22 (CO).

3-(2-Cyclopentylidene-2-phenyl-acetoxy)-1,1-dimethyl-pyrrolidinium bromide (**52**). 

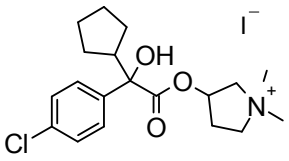
The solution of 2g (9.89mmol) cyclopentylidene-phenyl-acetic acid (**42**) in 9ml DMF was treated with 2.2g CDI in portions at rt. The mixture was left at rt for 30 minutes and 3.7g of **2** was added and the mixture was heated to 60°C for 36h. After cooling, 5ml water, 5ml toluene and 5ml 1N HCl were added. After stirring the phases were separated and the aqu. layer was washed with toluene, made alkaline with 1N NaOH and extracted with 2 5ml portions of toluene. The extract was evaporated to dryness to get 2.42g of the intermediate (MS, showinga mass of 286, confirms the structure of the intermediate, $M+H^+$). 2.22g of this intermediate was dissolved in 2g 1-propanol and treated with 7.78g of a 29% solution of methylbromide in 1-propanol. The mixture was stirred at 0°C for 2h and then heated to 60°C over the weekend. The mixture was cooled and evaporated to obtain 3.0g of **52** as an oil. ^{1}H-NMR (DMSO-d_6, 600MHz): 1.53 and 1.72 (m, 4H), 2.06 and 2.66 (m, 2H, C-4-H, pyrrolidinium); 2.17 and 2.82 (m, allylic CH_2 in cyclopentylidene moiety); 2.95 and 3.23 (s, 6H, N-CH_3); 3.65, 3.94 (m, C-2,5-H, pyrrolidine); 5.42 (br, CH-O); 7.20 (2H), 7,27 (1H), 7.35 (2H). IR (FT-IR microscope): 3419, 2957, 1710, 1631, 1475, 1455, 1283, 1203, 1152, 1070, 1035, 740, 703. MS: 300 (M^+), 645.6 ($2xM^+ + HCOO^-$). HR-MS, calculated for $C_{19}H_{26}O_2N$ 300.19581, found 300.19577. ^{13}C-NMR (600MHz, CD_3OD) 25.08, 26.14, 30.2, 33.68 & 34. 96 (allylic), 52.28 & 53.04 [$^+N(CH_3)_2$], 64.67 (^+N-CH_2), 70.14 (O-C-CH_2-^+N-), 71.51 (O-CH), 126.6 (p-C), 127.9 (m-C), , 128.2 (C-2), 128.8 (o-C), 138.4 (phenyl-1C), 165.1 (vinylic), 166.2 (CO).

1-Benzyl-3-(2-cyclopentyl-2-hydroxy-2-phenylacetoxy)-1-methylpyrrolidinium bromide (**54**). 

A solution of cyclopentyl-hydroxy-phenyl-acetic acid 1-methyl-pyrrolidin-3-yl ester (**3**) in toluene (18.3g, 49%, 30mmol, preparation see above) was diluted with 20g toluene and cooled to 0°C; benzylbromide (6.2g, 36mmol)

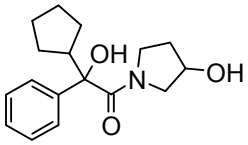
was added, the mixture was warmed to room temperature and stirred for 2h followed by the addition of 50ml isopropanol/water (2:1) and further stirring for 0.5h. Water was added to separate the phases. The aqueous phase was extracted with toluene and the aqueous phase was evaporated to dryness to get 13.57g of **54** as a white powder (mixture of diastereomers). MS: 294 (M^+), 484 ($M^+ + 2xHCOO^-$). IR (FT-IR microscope in transmission, cm^{-1}): 3364 (OH), 3089, 3060, 3005, 2956, 2868, 1734, 1448, 1232, 1168, 1073, 1031, 924, 733, 704. ^{1}H-NMR (DMSO-d_6, 600MHz, ppm): 1.1 – 1.6 (m, 8H), 2.0-2.3 and 2.6-2.7 (each m, each 1H, C-4-H pyrrolidinium), 1.8-1.9 (m, 1H, C-1-H cyclopentyl); 2.88, 2.92, 3.00, 3.01 (s, N-CH_3); 3.4-3.8 (m, 4H, C-2,5-H, pyrrolidinium); 4.6-4.7 (m, 2H, CH_2-Ph); 5.3-5.5 (m, 1H, CH-O); 5.8 and 5.9 (s, 1H, OH); 7.2-7.4 (m, 3H); 7.4-7.7 (m, 7H).

2-(4-Chloro-phenyl)-2-cyclopentyl-2-hydroxy-acetoxy]-1,1-dimethyl-pyrrolidinium iodide (**53**). A solution of 3.5g (4-Chloro-phenyl)-cyclopentyl-hydroxy-acetic acid **45** (13.8mmol) in 17ml of DMF was treated in portions

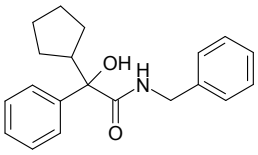


with 3.35g CDI (20mmol) at rt. After 30 more minutes 2.09 g 1-methyl-pyrrolidin-3-ol (20mmol) was added and the mixture was heated to 60°C for 18h. After cooling to rt 20ml water was added. The mixture was extracted with 20ml toluene and the organic phase was washed with water 3 times and partly evaporated to get 8.8g of an approximately 50% solution of *desmethyl-**53***. This solution was diluted with 12g acetone, cooled to 0°C and treated with 3.68g methyl iodide. The mixture was stirred for 1.5h at 0°C and 1h to 50°C. After the addition of 20mg of glycopyrrolate (**6**, finely ground; sometimes the addition crystalline material of a similar compound like here the DS, helps to crystallize the materials) the mixture was cooled to 15°C in 18h and stirring was continued for 48h. The solid material was filtered off and dried in vacuum to get 2.76g of **53** as the iodide salt. 1H-NMR (600MHz, DMSO-d_6, ppm): 1.20, 1.50, 1.60 (m, 8H, 4 CH_2-groups in cyclopentyl); 2.10 and 2.65 (br., 2H, C-4-H, pyrrolidinium); 3.15 and 3.19 (s, 6H, N-CH_3); 3.54, 3.65, 3.73, 3.86 (br., 4H, C-2,5-H, pyrrolidinium); 5.39 (br., CH-O); 5.93 (s, 1H, OH); 7.41 and 7.61 (d, 4H, C_6H_4-Cl). MS: 352/354 (for $C_{19}H_{27}ClNO_3$).

2-Cyclopentyl-2-hydroxy-1-(3-hydroxy-pyrrolidin-1-yl)-2-phenyl-ethanone (**55**). CPMA (**1**, 2g, 9mmol), dissolved in DMF (10ml) was activated with CDI (1.8g, 11mmol) by stirring at 5-25°C over 30minutes. After addition of pyrrolidin-3-ol (0.8g, 9.2mmol) the mixture was stirred for 18h at 25°C, quenched with 20ml of water and extracted with toluene (20ml). The organic phase was washed with water twice, dried over magnesium sulfate and evaporated to obtain the crude product (2.55g). LC-MS shows the presence of two isomeres (70%) and some by-products. HR-MS, calculated for M+H ($C_{17}H_{24}O_3N$) 290.17507, found 290.17511.



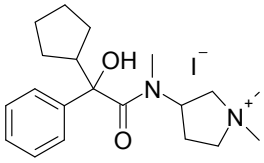
N-Benzyl-2-cyclopentyl-2-hydroxy-2-phenyl-acetamide (**56**). To the solution of **1** (4.0g, 0.018mol) in DMF (19g) was added within 15 min. CDI (3.18g, 0.0196mol) in portions. After 15 more minutes at 20°C, benzylamine (3.1g, 0.0289mol) was added and the temperature was raised to 30°C. After 3.5h water and TBME (57g each) were added and the mixture was stirred for 1h. The phases were separated. The aqueous phase was extracted with TBME and the combined organic phases were washed with 1M sulfuric acid (2x50ml), conc. sodium bicarbonate solution (2x50ml) and brine. The organic phase was evaporated to dryness and the solid crude product (6.03g) was recrystallized from toluene (21g) to give 5.05g (0.0163mol, 89.9%) of the pure amide **56**.



^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1.28 (m, 4H), 1.35-1.6 (m, 4H), 3.05 (m, 1H), 4.22 and 4.33 (ABX, J_{AB} = 15Hz, J_{AX}=6.0Hz, J_{BX}=6.6Hz, 4H), 5.61 (s, 1H, OH), 7.15-7.22 (m, 2H), 7.23-7.28 (m, 3H), 7.31-7.33 (t, 7.7Hz, 2H), 7.66 (d, 7.3Hz, 2H), 8.34 (t, 6.0Hz, 1H, NH). ^{13}C-NMR (100MHz, $CDCl_3$) δ = 25.99 (cyclopentyl-C3&4), 26.23 & 26.74 (cyclopentyl-C3 & -C5), 43.46 (N-C-Ph), 46.41 (cyclopentyl -C1), 79.95 (Ph-C-OH), 125.64, 127.39, 127.45, 128.32, 128.57, 128.62 (phenyl- & phenyl'- o-, m-, p-C), 138.2 & 142.7 (phenyl- & phenyl'- C1), 174.1 (CO).^{13}C-NMR (150 MHz, DMSO-d_6): δ = 25.59, 25.75, 25.94, 26.43, 42.10 (CH_2Ph), 46.31, 79.44 (C-O), 125.75, 126.92, 127.54, 128.05, 139.70, 144.00, 173.95 (C=O). FTIR-microscope in transmission: 3389 (NH, OH), 3303 (br), 3087, 3062, 3023 (arom. CH), 2954, 2914, 2869 (aliph. CH), 1662 (CO-N), 1525 (Amid II), 1494, 1450, 1429, 1368, 1328, 1273, 1217, 1178, 1097, 1074, 1037 (C-O), 730, 698. MS ($C_{20}H_{23}NO_2$, exact mass

309.1729) – ES^+: 292 (40%, $M+H^+-H_2O$), 310 (100%, $M+H^+$), 619 (10%, $2M+H^+$), 641 ($2M+Na^+$); ES^- : 308 (M-H). HR-MS: calculated for $C_{20}H_{24}O_2N$ ($M+H^+$) 310.1806, found 310.18008 (-0.2ppm).

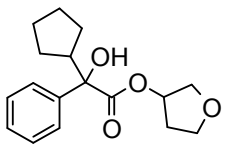
3-[(2-Cyclopentyl-2-hydroxy-2-phenyl-acetyl)-methyl-amino]-1,1-dimethyl-pyrrolidinium iodide and bromide (**57**). CPMA **1** (11.5g ,52mmol) was dissolved in 48 ml of DMF and treated at rt with 11.9g (73mmol, 1.4eq) CDI in 6 portions. After stirring for 30 minutes, 8.574g (73mmol) of N,N'-dimethyl-3-amino-pyrrolidine were added at and the mixture was heated for 18h at 60°C. After cooling to rt 25ml of water was added and the mixture was made acidic (pH<2) by adding 24ml of 1N HCl and washed 3 times with TBME. The aqu. phase was then made alkaline by adding 35ml of 1N NaOH and extracted with 25ml TMBE twice. The combined extracts were evaporated to get 1.72g of 2-cyclopentyl-2-hydroxy-N-methyl-N-(1-methyl-pyrrolidin-3-yl)-2-phenyl-acetamide (pre-**57,** two isomers by HPLC-MS, m/z = 317; HR-MS, calculated for $M+H^+$ ($C_{19}H_{29}O_2N_2$) 317.2224, found 317.2223).



1.50g (4.7mmol) of pre-**57** was dissolved in 5ml of acetone and treated at 0°C with 1.01g of methyliodide. After stirring over night at 25°C the mixture was cooled to 5°C in 18h to obtain a colorless suspension. The solid was filtered, washed with acetone and dried in vacuum to obtain 0.672g (1.47mmol, 31%) of **57-iodide**. The NMR-spectrum shows broad peaks. HR-MS: calculated for M^+ ($C_{20}H_{31}O_2N_2$) 331.2380, found 331.23810.

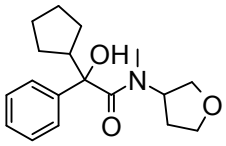
0.22g (0.7mmol) of pre-**57** was dissolved in 3ml of acetone, cooled to 0°C and treated with 0.34g of a 29% solution (1mmol) of bromomethane in 1-propanol. After 1.5h the mixture was warmed to 25°C and stirred over night. The mixture was then cooled to 5°C over 18h whereupon precipitation occurs. The solid was filtered, washed with acetone and dried in vacuum to obtain 0.149g (0.36mmol, 52%) of **57-bromide**.

Cyclopentyl-hydroxy-phenyl-acetic acid tetrahydro-furan-3-yl ester (**58**):



Cyclopentylmandelic acid **1** (2g, 0.009mol) in DMF (10ml) was treated with CDI (2.03, 0.012mol) at 12°C. After 30 minutes at rt, 3-hydroxy-tetrahydrofuran **31** (1.64g, 0.018mol) was added and the mixture was heated to 45°C over night. Water and THF (10ml each) were added and the resulting mixture was heated to 40°C for 18h to destroy unreacted imidazolide **7**. After cooling to rt, the mixture was extracted with TBME, the extract was washed with water, sodium bicarbonate and sodium chloride solution and evaporated to obtain 1.8g of **57** as a colorless oil, composed of an inseparated mixture (~1:1) of diastereomeres. ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1.1-1.6 (m, 8H), 1.77, 1.87, 2.08, 2.13 (each m, each 0.5H), 2.86 (m, 1H), 3.5-3.8 (m, 4H), 5.22 (m, 1H), 5.67 (s, 1H, OH), 7. 26 (m, 1H), 7.34 (m, 2H), 7.58 (m, 2H); FTIR-microscope in transmission: 3513 (OH), 2955, 2869, 1725 (CO), 1447, 1252, 1174, 1107, 1074, 700. HR-MS: calculated for $C_{17}H_{23}O_4$ ($M+H^+$) 291.15909, found 291.15881; calculated for $C_{17}H_{26}O_4N$ ($M+NH_4^+$) 308.18564, found 308.18539; calculated for $C_{17}H_{22}O_4Na$ ($M+Na^+$) 313.14103, found 313.14066.

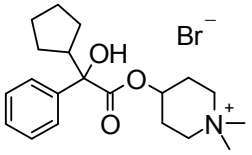
2-Cyclopentyl-2-hydroxy-N-methyl-2-phenyl-N-(tetrahydro-furan-3-yl)-acetamide (**59**):



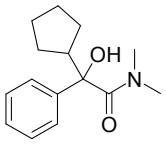
Cyclopentylmandelic acid **1** (9.5 g, 43 mmol), dissolved in DMF (45 mL), was treated with an excess of 1,1'-carbonyldiimidazole (9.62 g, 59.3) at 20°C. After addition of N-methyltetrahydrofuran-3-yl-amine **33** (6.0 g, 59.3 mmol), the mixture was heated to 40°C within 1 hour and stirred overnight at this temperature. The mixture was then cooled to room temperature and water (60 mL) was added. The mixture was extracted with toluene (70 mL) and the toluene extract was washed with water (35 mL) 3 times. The organic phase was concentrated to obtain a crude product as a solid foam. TLC and HPLC-analyses revealed a mixture of products showing two isomeres of **59** by MS. By-products were identified as CPMA (**1**), CPMA-dimethylamide (**61**), the dimeric ester of CPMA. The mixture was separated by preparative TLC using silica as stationary phase and a mixture of toluene, ethyl acetate and methanol (150:90:10) as eluent. From the fractions the structure of **59** could be confirmed by HR-MS, calculated/found/for formula: 304.1913/304.1909/$M+H^+$

($C_{18}H_{26}NO_3$), 286.1807/286.1802/M+H^+-H_2O ($C_{18}H_{26}NO_2$), 326.1732/336.1724/M+Na^+ ($C_{18}H_{25}O_3NNa$). For the spiking experiment however the crude product was used.

4-(2-Cyclopentyl-2-hydroxy-2-phenyl-acetoxy)-1,1-dimethyl-piperidinium bromide (**60**). Following the general procedure, 2g CPMA (**1**) in DMF was activated with CDI and reacted with N-methyl-4-hydroxypiperidin (**35c**) at 60°C for 18h. After usual workup (water, toluene), the intermediate in 1-propanol was reacted with excess methylbromide (**4**) at 0 – 60°C. After reacting over the weekend, the mixture was cooled and the solid formed was filtered and dried to get 3.09g of **60**. 1H-NMR (600MHz, DMSO-d_6, ppm): δ = 1.19, 1.26, 1.41, 1.51, 1.59, 1.81, 2.18 (br., 12H), 2.96 (br. t, 1H, C-1-H, cyclopentyl), 3.14, 3.20 (s, 6H, N-CH_3), 4.93 (s, CH-O), 5.69 (s, OH), 7.26 (br., 1H), 7.35 (br., 2H), 7.64 (br., 2H). ^{13}C-NMR (100MHz, D_2O) δ = 23.97, 24.32, 24.69, 26.11, 26.29, 26.68, 45.16, 48.64 & 54.65 [N-$(CH_3)_2$], 58.41 & 58.13 [$(-CH_2)_2$N], 66.08 (C-O), 80.75 [Ph-C(OH)], 126.17 (phenyl-o-C), 128.35 (phenyl-p-C), 128.81 (phenyl-m-C), 141.43 (aryl-C-1), 174.5 (CO), (besides the peaks of 1-propanol: 9.66, 25.86, 63.66). MS: 332 (M^+), 422 (M^+ + 2 $HCOO^-$) accounts for $C_{20}H_{30}NO_3$. HR-MS: calculated for $C_{20}H_{30}O_3N$ 332.22202, found 332.22192 (-0.3ppm).

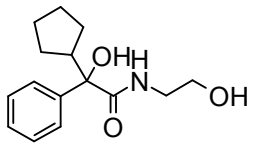


2-Cyclopentyl-2-hydroxy-N,N-dimethyl-2-phenyl-acetamide (**61**). The solution of 10.63g (0.047mol) **1** in DMF (50ml) was treated in portions with 9.5g (0.057mol) CDI at 18-22°C. After stirring for 30 minutes, 6.4g of a 40% aqueous solution of diemthylamine (0.57mol) were added and the mixture was stirred at 18°C for one hour and at 60°C for another hour. The mixture was cooled, 50ml water were added and stirring was continued for 30 minutes followed by extraction with TBME (2x40ml). The combined extracts were washed with water and evaporated to dryness to obtain **61 (**8.7g, 0.035mol, 75%) as a colorless solid.



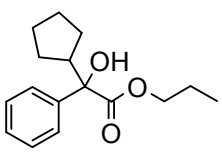
^{1}H-NMR (600 MHz, $CDCl_3$): δ = 1.4 – 1.88 (m, 8H), 2.91 (s, 3H), 2.98 (s, 3H), 2.98-3.07 (m, 1H), 5.30 (s, br., 1H, OH), 7.25-7.30 (m, 1H), 7.33-7.38 (m, 2H), 7.42-7.46 (m, 2H). IR (FTIR-microscope, cm^{-1}): 3384, 2956, 2868, 1620, 1496, 1446, 1398, 1353, 1271, 1251, 1150, 744, 704. MS: m/z = 248 ($M+H^+$), 230 ($M+H^+-H_2O$). HR-MS: calculated for $C_{15}H_{22}O_2N$ ($M+H^+$) 248.16451, found 248.16441 (-0.4ppm). ^{13}C-NMR (600MHz, CD_3OD): 25.16, 25.49, 26.41, 26.95, 36.12 & 36.66 [$^{+}N(CH_3)_2$], 47.72, 80.31 (C-2), 124.55, 126.32, 127.43, 142.30, 174.1 (CO).

2-Cyclopentyl-2-hydroxy-N-(2-hydroxy-ethyl)-2-phenyl-acetamide (**62**):



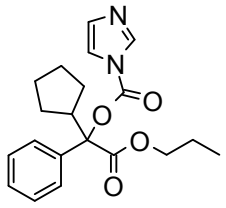
After activation of **1** (4g, 0.018mol) with CDI (4.08g, 0.025mol) in DMF (20ml), ethanolamine (2.4g, 0.039mol) was added and the mixture was stirred at 40°C over night. The mixture was quenched with water and extracted with TBME. The extract was washed with 10% aq. sodium bicarbonate and brine and evaporated to obtain 4.1g (0.0156mol, 86%) of crude **62** which was recrystallized from toluene. ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1,23 (m, 2H), 1.3-1.6 (m, 6H), 2.95 (quintet, 1H), 3.05 and 3.15 (each m, each 1H, CH_2-N), 3.36 (m, 2H, CH_2-O), 4.68 (t, 1H, OH), 5.56 (s, 1H, OH), 7.20 (t, 1H), 7.29 (t, 2H), 7.57 (d, 2H), 7.74 (t, 1H, NH); FTIR-microscope in transmission: 3351 (N-H, O-H), 2952, 2868, 1659 (N-C=O), 1544 (amide II), 1494, 1469, 1446, 1425, 1339, 1280, 1243, 1168, 1076 (C-OH), 692; HR-MS, calc. for M+H = $C_{15}H_{22}O_3N$ 264.15942, found 264.15938 (-0.14ppm), calc. for M+Na = $C_{14}H_{21}O_3NNa$ 286.14137, found 286.14125 (-0.25ppm). ^{13}C-NMR (600MHz, CD_3OD): 25.35, 25.49, 25.57, 26.05, 40.9, 45.9, 60.0, 79.4, 125.3, 126.5, 127.3, 142.8, 175.6.

Cyclopentyl-hydroxy-phenyl-acetic acid propyl ester (**1c**) and **Imidazole-1-carboxylic acid cyclopentyl-phenyl-propoxycarbonyl-methyl ester** (**65**): CPMA **1** (2g, 9mmol) in DMF (10ml) was treated with CDI (2.04g, 12.6mmol) at 70°C for 18h. 7ml 1-propanol was added and the mixture was kept at 70°C for additional 24h. After cooling and the addition of water, the product was extracted with TBME. The crude product after evaporation



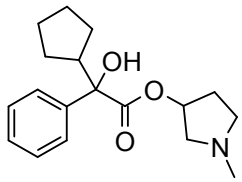
(2.18g) was purified by flash chromatography using silica eluting with heptane with increasing amount of ethyl acetate. 1.58g of **1c** and 0.19g of **65** were obtained as liquid compounds.

1c: R_f = 0.59 (silica, heptane/ethyl acetate = 4:1), ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 0.83 (t, 3H), 1.1-1.6 (m, 10H), 2.88 (quint., 1H), 3.99, 4.03 (ABXY, 2H), 5.61 (s. 1H, OH), 7.25 (t, 1H), 7.33 (t, 2H), 7.58 (d, 2H); FTIR-microscope in transmission: 3511 (OH), 2961, 2869, 1721 (C=O), 1615, 1601, 1493, 1448, 1378, 1348, 1243, 1193, 1174, 1118, 1105, 1071, 1058, 1036, 935, 731, 700;



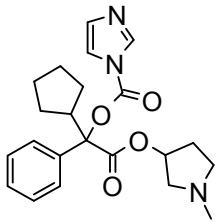
65: R_f = 0.30 (silica, heptane/ethyl acetate = 4:1), ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 0.82 (t, 3H), 1.1-1.5 (m, 6H), 1.54 (m, 2H, C-CH_2-Me), 1.7 (m, 1H), 1.82 (m, 1H), 3.21 (quintet, 1H), 4.09 (m, 2H, O-CH_2-C-Me), 6.92 (s, 1H), 7.31 (s, 1H), 7.38 (d, 2H), 7.44 (t, 1H), 7.49 (t, 2H), 8.00 (s, 1H). FTIR-microscope in transmission: 3143 (Imidazol-C-H), 3031 (arom. CH), 2965, 2874 (aliph-CH), 1751 (C=O), 1584, 1466, 1370, 1287, 1270, 1237, 1217, 1097, 1048, 937, 734, 709; MS ($C_{20}H_{24}N_2O_4$, 356.1736), found 357.1 (M+H), 289.1 (M – $C_3H_3N_2$), 261.1 (M – CO-Im), 175 (100%, Ph-C(OH)-cyC_5H_9).

Cyclopentyl-hydroxy-phenyl-acetic acid 1-methyl-pyrrolidin-3-yl ester (**3**) and **imidazole-1-carboxylic acid cyclopentyl-(1-methyl-pyrrolidin-3-yloxycarbonyl)-phenyl-methyl ester** (**67**). Attention: Compound **3** is a compound causing reversible exogeneous psychosis; preparation and handling needs to be performed with extreme care.



CPMA **1** (1g, 4.5mmol) in DMF (5ml) was activated with CDI (1.84g, 11.35mmol) at 70°C for 20h. 1-Methyl-pyrrolidin-3-ol (**2**, 1.84g, 18.1mmol) was added and stirring at 70°C was continued for 24h. The mixture was treated with water and extracted with TBME. The organic phase was washed with water and aqu. sodium carbonate solution, dried over sodium sulfate and evaporated to obtain 1.5g of the crude product consisting of **3** (about 75%) and a mixture of isomers of **66** (about 20%). The components were separated by flash chromatography (silica, eluting with TBME/ethyl acetate (1:1) and ethyl acetate/methanol (1:1).

67: ^{1}H-NMR (600 MHz, DMSO-d_6): δ = 1.0-1.8 (m, 10H), 2.08 (m, 1H), 2.18 and 2.24 (s, ~1:1, 3H, N-CH_3), 2.4-2.8 (m, 4H), 4.97 (br., 1H), 6.92 and 7.0 (s, 1H), 7.28 (s, 1H), 7.38 (m, 2H), 7.43 (m, 1H), 7.48 (m, 2H), 7.99 (s, 1H). FTIR-microscope in transmission: 3384, 3138, 3032, 2954, 2870, 2785 (N-CH), 1749, 1602, 1467, 1449, 1369, 1333, 1271, 1237, 1097, 1069, 963, 773, 709. LC-MS, 398.2 (20%, M+H), 330.2 (100%, M+H-$C_3H_4N_2$).



Spiking experiments

Crude glycopyrrolate **5** (5.19g) was mixed with 50mg of each **49**, **52**, pre-**57**, **60** and **61** and the mixture was dissolved in 1-propanol (47g) at 65°C. The solution was cooled to 50°C, seeded with 15mg of **6** and slowly cooled during 18h to 15°C. The solid thus obtained was filtered and dried in vacuum to get 3.91g (75%) of **B6** (only once recrystallized).

In a similar experiment, 5.19g of **5** was recrystallized in the presence of 50mg of each **48**, **54**, **55**, **58** and **62** together with a mixture of **53** and its desmethyl precursor pre-**53** to get 4.78g (92%) of **6**. The crystallized materials as well as the corresponding mother liquors were analyzed according to the drug substance release testing method. The analytical results are summarized in table 7.

In three other experiments the depletion factors of **56**, **57** and **59** were determined (see table 8); the isomers of **57** were quantified by mass specific detection in an LC-MS experiment.[16]

For this compound the depletion factor can be determined also simply by calculating DF = c^0/c^K = 0.064/0.003 = 21 for one isomer and DF = c^0/c^K = 4.25/0.11 = 39 for the other isomer which matches partly with the calculation taking the concentrations in K and ML into account.

For three of the precursors of the quaternary ammonium salts the depletion factors were established. These neutral molecules are well soluble in 1-propanol, see table 8.

Table 7: composition of crystalline **6** ("K") and the corresponding mother liquor (ML) obtained by recrystallization of **5** where the mentioned impurities were spiked and calculation of the respective depletion factors (DF).

Compound	K (y=0.75)	ML	**DF**
6	99.833	64.099	
1	<0.015	0.118	>3
49	0.037	3.473	24
52	0.056	7.483	34
60	0.015	4.086	69
61	<0.015	10.215	>171

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

Compound	K (y=0.92)	ML	**DF**
6	97.020	41.137	
53	1.920	2.373	1
pre-**53**	0.139	39.537	23
48	0.044	5.610	11
62			
54	<0.015	0.876	>6
	<0.015	1.013	>6
55	0.036	0.649	2
	<0.015	0.568	>4
58	<0.015	1.589	>9

Deriving the formula for the depletion factor:

$$DF = \frac{c^0}{c^K} = \frac{m^0}{M*c^K} = \frac{c^K*m^K + c^{ML}*m^{ML}}{M*c^K} = \frac{c^K*M*y + c^{ML}*M(1-y)}{M*c^K} = \frac{c^K*y + c^{ML}*(1-y)}{c^K} = y + \frac{c^{ML}*(1-y)}{c^K}$$

c^0: content of the impuritiy before crystallization (area-% in HPLC)

c^K: content of the impuritiy in the recrystallized material,

m^0: amount of the impurity in the batch before crystallization,

M: amount of batch before crystallization,

c^{ML}: content of the impuritiy in the mother liquor,

y: yield of the crystallization ($0<y<1$), $y = m^K/M$

m^K: amount of recrystallized material,

m^{ML}: amount of mother liquor (evap. Residue); $m^K + m^{ML} = M$

By this calculation the DF is also independent on an eventually incorrect amount or purity of the compound spiked for the crystallization experiment.

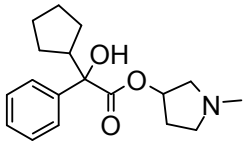
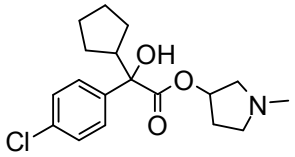
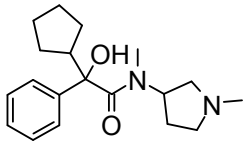
Table 8: experiments to determine the depletion factors of **56**, **57**, **59**

	by-product [g]	**5** [g]	1-PrOH [g]	%a of by-product in solution	%a of by-product in cryst. **6**	%a in mother liquor	Yield of **6**	DF
56	0.036	3.6	35	n.d.	<0.05%	6.1%	2.66g, 74%	37
57	0.022	2.2	14	0.064/4.25%*	0.003/0.11%*	0.46/14.7%*	1.90g, 86%	≥20
59	0.050	4.8	43	0.1-0.2%**	<0.05%	0.3-0.9%	3.41g, 71%	>2

*LC-MS/mass-specific detection because peaks under main (see below); **several peaks

	area for mass			**content isomer 331**	
	317/319	**330/332 1st**	**330/332 2nd**	**1st**	**2nd**
5 + 1% **57**	5.46E+07	3.64E+04	2.43E+06	0.064%	4.25%
crystallized **6**	4.43E+07	1.36E+03	4.93E+04	0.003%	0.11%
mother liquor	2.56E+07	1.37E+05	4.43E+06	0.455%	14.68%

Table 9: depletion of glycopyrrolate precursor and selected analogues

Structure of 3 or analogue			
#	**3**	pre-**53**	pre-**57**
rRT	1.07	1.29	1.04; 1.07
DF	>5*	23	>25; >67

*based on the finding of 0.41% B3 in a mother liquor, <0.05% in drug substance and a yield of 42%.

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

n.d. 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

References and notes

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[10] The components can easily be assigned by comparison with the GC-retention times of original samples but also by taking ^{13}C-NMR spectra. In case the starting material **9** contains some tribromobutanol **9a**, the reaction mixture will contain N,N'-dimethyl-3-aminopyrrolidine **30**.

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[16] It needs to be pointed out, that the linearity of the responses as well as the ratios (contents) for the mass specific detection is not established. However the mere comparison of the chromatograms with the mass specific detection shows clearly the huge depletion of the potential impurity **57** during crystallization of the drug substance.