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Development of an efficient and scalable process for rocuronium bromide: A neuromuscular blocking agent

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ABSTRACT

An efficient, scalable and high yielding process is developed for a neuromuscular blocking agent Rocuronium bromide **1** by studying various process parameters. The process involves simple work up and obviates column chromatography purification and usage of commercially unacceptable solvents.

Key words: Rocuronium Bromide, neuro muscular blocking agent, diepoxide, acetylation, allylation.

INTRODUCTION

Rocuronium bromide[1] is a non-depolarizing neuromuscular blocking agent[2] used as anaesthetic in surgeries. The reported synthesis[3, 4] of Rocuronium bromide 1 involved base-catalyzed condensation of diepoxide 2 and pyrrolidine in methanol in the presence of sodium hydroxide followed by in-situ reduction of keto intermediate to give the corresponding secondary alcohol 3. Opening of epoxide ring of 3 using morpholine and subsequent selective acetylation of resulted 2-morpholinyl-3, 17-dihydroxy steroidal derivative 4 furnished 17-acetoxy derivative 5 which on reacting with allyl bromide provided Rocuronium bromide 1 in 19.6% overall yield. Several column chromatography purifications and usage of commercially unacceptable solvents made this reported process less attractive and commercially non- viable. Now we worked on this process and made appropriate process modifications, obviating the column chromatography purifications and replacing some of the solvents with commercially acceptable ones, rendering the process effective and scalable. The details are described in this paper.

EXPERIMENTAL

The ^{1}H-NMR spectra were recorded in $CDCl_3$, on a Varian Gemini 400 MHz FT NMR spectrometer and the chemical shifts are reported in δ ppm relative to TMS. The Mass spectra (70 eV) were recorded on HP-5989 LCMS spectrometer. The CHN analysis was carried out on a Perkin-Elmer model 2400S analyzer.

Preparation of (2a, 3a, 5a 16b, 17b) 2,3-epoxy-16-(1-pyrolidinyl)-androstan-17-ol 3

A mixture of 2 (12.0 kg, 34.68 mol) methanol (120 L) and sodium hydroxide (1.94 kg, 48.5 mol) was stirred at 40°C for 10 minutes and pyrrolidine (11.5 L, 137.8 mol) was added to the

reaction mixture. Solution was heated under reflux for 20 minutes, cooled to 5°C and sodium borohydride (4.0 kg, 105.8 mol) was charged at below 10°C. The solution was stirred at 30-35°C for 1 hr and the reaction mass was quenched into chilled water (480 L). Obtained solid was dissolved in dichloromethane (36 L), organic layer was separated and washed with NaCl solution (1.2 kg) in (13.2 L). The solution was filtered through celite and the celite was washed with dichloromethane (12 L). Organic layer was concentrated, acetone (60 L) was charged to the crude and solution was stirred at reflux for 30 minutes, cooled to 32-35°C and further stirred for 1 hr. Solid was filtered and washed with acetone (19.2 L) to provide compound 3. (Yield: 9.6 kg, HPLC purity: 98.4%); ^{1}H NMR ($CDCl_3$, δ ppm) : δ 3.35 (d, 1H), 3.1 (br q, 2H), 2.9 (q, 1H), 2.7 (t, 2H), 2.45 (t, 2H), 1.85 (m, 5H), 1.6(m, 10H), 1.3 (m, 10H), 0.8 (m, 4H); Mass: 359 (M^{+1}); C H N Analysis Calcd. for $C_{23}H_{37}NO_2$; C, 76.83; H, 10.37; N, 3.90% Found : C, 76.42; H, 10.11; N, 3.68%; $[\alpha]^{20}_D$: +33° (C=1.05 $CHCl_3$).

Preparation of (2b, 3a, 5a 16b, 17b) 2-(4-morpholinyl)-16-(1-pyrolidinyl)-androstane-3, 17-diol 4

A mixture of compound 3 (11 kg, 30.64 mol) and morpholine (63.8 L, 729.5 mol) in water (6.6 L) was heated under reflux for 68 hrs. Reaction mass was cooled to 25°C, quenched into water (165 L) and stirred for 1.5 hrs. Solid was filtered and washed with water (22 L). The wet solid was slurried in acetone (55 L), filtered, washed with acetone (11 L), recrystallized from methanol (33 L) to give compound 4 as a white crystalline powder. (Yield: 9.9 kg, HPLC purity: 97.8%); ^{1}H NMR ($CDCl_3$, δ ppm) : δ 3.85 (q, 1H), 3.65 (br t, 4H), 3.4 (t, 1H), 2.9 (q, 1H), 2.4 (m, 9H), 1.9 (m, 4H), 1.7 (s, 6H), 1.5 (m, 10H), 1.2 (m, 2H), 0.8 (m, 8H); Mass: 446 (M^{+1}); C H N Analysis Calcd. for $C_{27}H_{46}N_2O_3$; C, 72.60; H, 10.38; N, 6.27% Found : C, 72.42; H, 10.31; N, 6.20 %; $[\alpha]^{20}_D$: +86.5° (C=1.02 $CHCl_3$).

Preparation of (2b, 3a, 5a 16b, 17b) 2-(4-morpholinyl)-16-(1-pyrolidinyl)-androstane-3, 17-diol-17-acetate 5

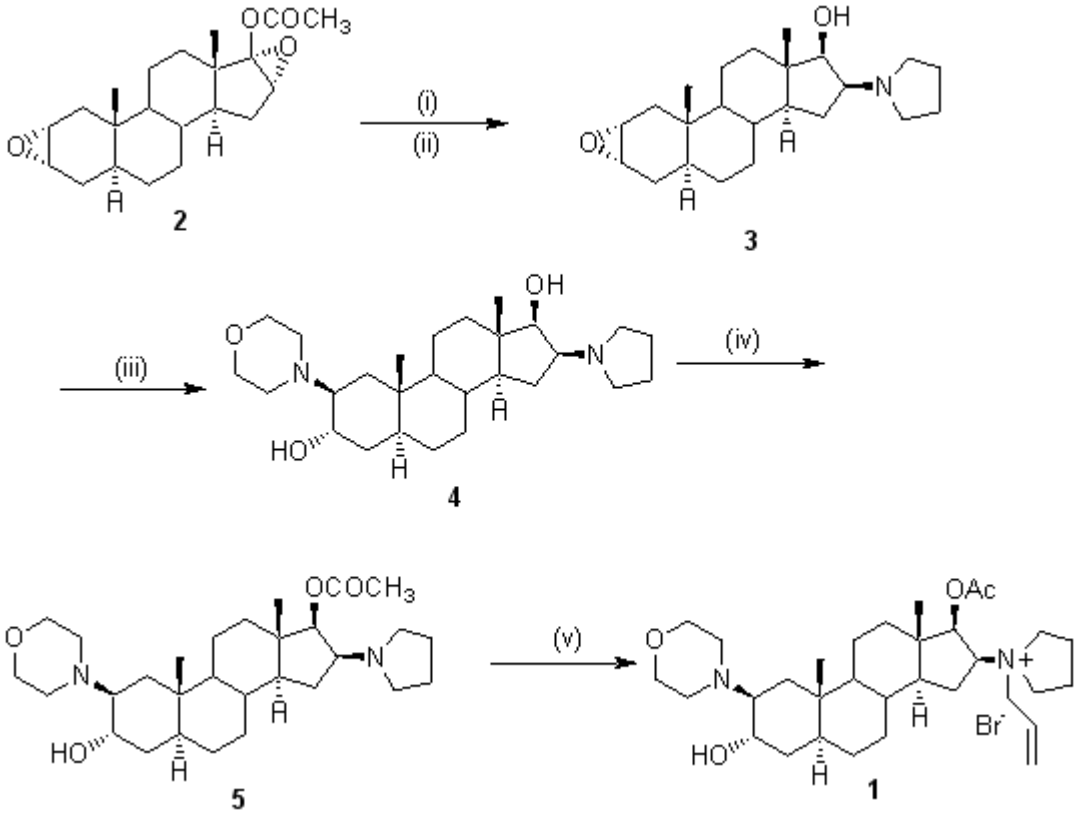
Acetyl chloride (1.67 L, 23.5 mol) was added to a solution of compound 4 (10 kg, 22.4 mol), in dichloromethane (300 L) and reaction mixture was stirred for 3 hrs. Solvent was stirred under reduced pressure and dichloromethane (50 L) was charged to the resultant crude. Solution was cooled to 5°C, stirred for 1 hr. and unreacted compound 4 was filtered off. Filtrate was diluted with dichloromethane (100 L) and water (40 L), p^H of the solution was adjusted to 6.5-7.0 with 5% sodium carbonate solution. Solution was stirred for 20 minutes and organic layer was separated. Organic layer washed with water (80 L), dried and concentrated under vacuum and crude was recrystallized from acetonitrile (50 L). The dried solid was taken in acetone (30 L) and heated to reflux for 30 minutes. The homogeneous solution was cooled to 0°C. Solid was filtered, washed with chilled acetone (10 L) and dried the solid at 55°C for 6 hrs to provide compound 5. (Yield: 7.8 kg, HPLC purity: 98.5%); ^{1}H NMR ($CDCl_3$, δ ppm) : δ 3.7 (s, 4H), 3.2 (d, 1H), 2.6 (t, 4H), 2.4 (m, 4H), 2.15 (s, 3H), 1.75 (m, 4H), 1.6(m, 6H), 1.2 (m, 13H), 0.8 (m, 9H); Mass: 488 (M^{+1}); C H N Analysis Calcd. for $C_{29}H_{47}N_2O_4$; C, 71.27; H, 9.90; N, 5.73% Found : C, 71.05; H, 9.73; N, 5.64%; $[\alpha]^{20}_D$: +53.2° (C=1.03 $CHCl_3$).

Preparation of 1-[17b-(acetyloxy)-3a-hydroxy-2b-(morpholin-4-yl)-5a-androstan-16b-yl]-1-(prop-2-enyl) pyrolidinium bromide 1

A mixture of 5 (15 kg, 30.7 mol), dichloromethane (375 L), allyl bromide (27.6 L, 319.1 mol) and water (7.5 L) was stirred for 12 hours. Volatiles were evaporated under reduced pressure and dichloromethane (75 L) was charged to the resulted crude and solvent was distilled under reduced pressure. Obtained crude was purified with the mixture of dichloromethane (75 L) and methyl t-butyl ether (450 L). Solid was filtered, washed with methyl t-butyl ether (75 L), dried for 30 minutes, and this operation repeated. Solid was dried at 40°C for 7 hours under vacuum (10 mbar) to give compound 1. (Yield: 16.9 kg, HPLC purity: 99.8%); ^{1}H NMR ($CDCl_3$, δ ppm) : δ 6.18 (dd, 1H), 5.72 (m, 2H), 5.22 (d, 1H), 4.35 (m, 1H), 4.17 (m, 1H), 3.88 (m, 4H), 3.70 (m, 4H), 2.62 (m, 4H), 2.46 (m, 2H), 2.29 (m, 1H), 2.28 (m, 4H), 2.24 (s, 3H), 1.82 (m, 4H), 1.59 (m, 5H), 1.22 (m, 8H), 1.03 (m, 1H), 0.85 (m, 7H); Mass: 609 (M^{+1}); C H N Analysis Calcd. for $C_{32}H_{53}BrN_2O_4$; C, 63.04; H, 8.76; N, 4.59% Found : C, 62.91; H, 8.69; N, 4.48%; $[\alpha]^{20}_D$: +18.4° (C=1.03 $CHCl_3$).

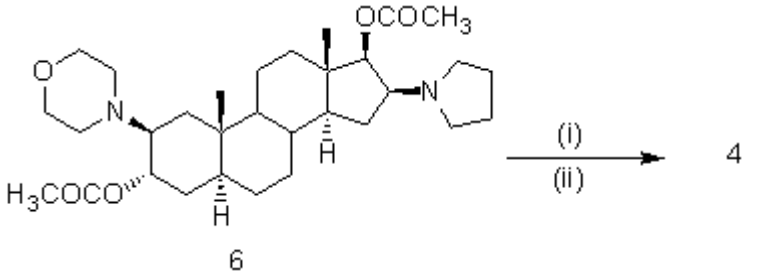
Preparation of (2b, 3a, 5a 16b, 17b) 2-(4-morpholinyl)-16-(1-pyrolidinyl)-androstane-3, 17-diol 4 from the mother liquor of compound 5.

To the crude of the filtrate of compound 5 (90 g, 0.16mol), added 10 % aq. Potassium hydroxide (0.45 L), methanol (0.9 L) and the mass was stirred at reflux for 5 hours, cooled to 30°C. Solid was filtered, washed with methanol (0.2 L). Recrystallized the resultant solid in methanol (0.45 L) to give the pure product 4



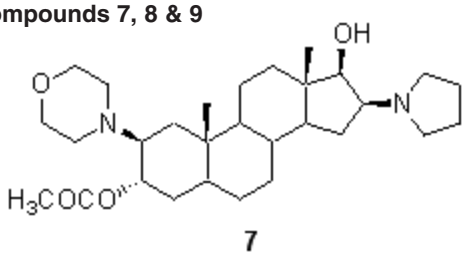
Reagents and conditions: (i) Pyrollidine, Methanol, NaOH, reflux (ii) $NaBH_4$, Methanol, 30-35°C (iii) Morpholine, Water, reflux (iv) Acetyl chloride, Dichloromethane, 25-30°C (iv) Allyl bromide, Dichloromethane, 25-30°C

Scheme 1



Reagents and conditions: (i) aq. KOH, methanol, reflux (ii) Methanol (ii) Methanol, reflux

Compounds 7, 8 & 9



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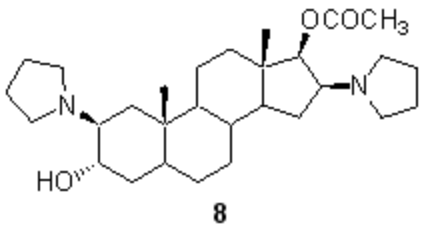

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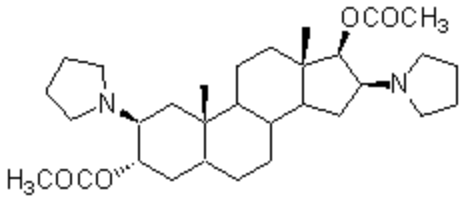

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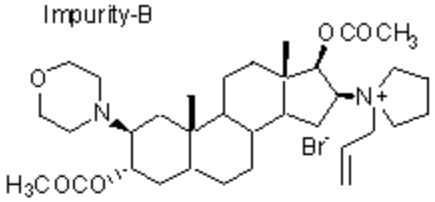
European pharmacopoeia Impurity Structures

Impurity-B



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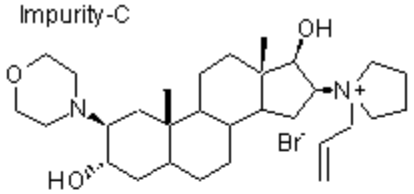
Impurity-C



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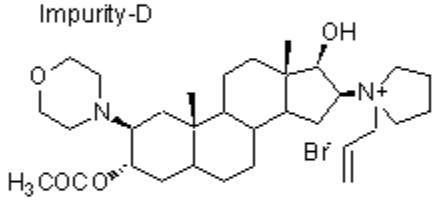
Impurity-D



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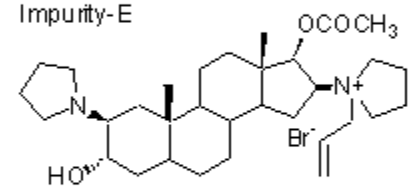
Impurity-E



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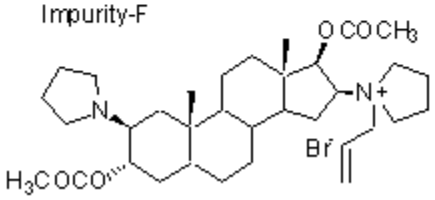
Impurity-F



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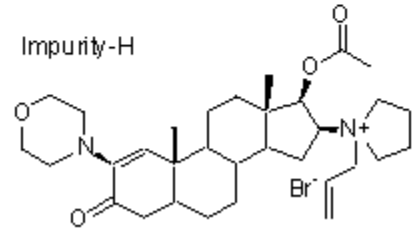
Impurity-H



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Compound 4= Impurity A and Compound 3= Impurity G

Scheme 2

RESULTS AND DISCUSSION

We made a detailed study of process parameters in each stage of the synthetic sequence and optimized the process conditions (Scheme 1). Conversion of diepoxy ester 2 in to epoxy alcohol derivative 3 involved nucleophilic addition of pyrrolidine at C-16 –OH and concomitant ester hydrolysis followed by $NaBH_4$ reduction of intermediate ketone. While quenching the $NaBH_4$ following reported process, lot of emulsion was formed making the filtration operation very difficult. Extra water washing and usage of celite pad has alleviated the emulsion problem enabling the smooth filtration of reaction mass.

Temperature of 32-35ºC was found to be an apt range of temperature for the isolation of the product in better yield and quality. At room temperature, significant amount of emulsion in the reaction mass hindered the filtration through the filter pad.

Transformation of compound 3 to 4 involved nucleophilic addition of morpholine at C-2 in refluxing aqueous media. In this stage we have observed that, distillation of morpholine as per the literature procedure resulted in enhanced levels of impurities. Instead, pouring the reaction mass in to water at ambient temperature, isolation of product and crystallization from acetone yielded the compound 4 in 65-73% with 96-98% purity.

As per the chemistry involved, an equimolar ratio of the acetyl chloride is required for the conversion of 4 to 5. When we conducted the acetylation reaction of 4 using 1.2 moles of acetyl chloride, following literature process, significant amount of diacetyl derivatives 6 is formed as by-product. We have optimized the mole ratio of acetyl chloride and reaction time to minimize the formation of diacetyl product 6.

The acetylation reaction was monitored by online HPLC (Table 1). The HPLC data indicated that, using 1.05 molar ratio of acetyl chloride the reaction yielded 76% of desired product 5 along with 8% of diacetyl derivative and 15% of unreacted

Table 1: HPLC data of acetylation reaction using 1.05-mole ratio of acetyl chloride

Exp. No	Reaction Mass maintenance time (hr).	4 (%)	5 (%)	6 (%)
4	2	15.02	76.74	8.16
5	4	15.08	76.46	8.37
6	5	15.21	76.33	8.39
7	8	15.23	76.28	8.41
8	10	15.28	76.25	8.41

Table 2 : Acetone[a] purification HPLC Data

Exp.	Before Purification		After Purification		Yield
No	4 (%)	3 (%)	4 (%)	3 (%)	
1	75.51	12.15	96.51	0.91	66%
2	93.18	3.04	98.52	0.14	70%
3	82.16	5.65	96.92	0.27	68%

[a] Acetone qty. 5 times to the batch size and the temperature 25-30°C

Table 3: The final quality of rocuronium bromide drug substance

Exp.	Purity by HPLC (1) (%)	Content of other impurities (%)							
		3	5	10	11	12	13	14	15
9	99.8	ND	0.02	0.01	0.01	0.01	ND	ND	0.01
10	99.82	ND	0.02	0.02	0.01	ND	0.03	ND	ND
11	99.81	ND	ND	0.03	0.04	0.01	0.01	0.03	0.005

4. Further, similar conversion trend is observed irrespective of the reaction time. Unreacted diol 4 was isolated from the reaction mass with appropriate work up. The mother liquor of compound 5 contains excess of compound 6 and a minimum level of compound 5. The filtrate was evaporated and heated with aqueous alkali and methanol to provide compound 4, which was further purified from methanol yielded the diol compound 4 in 60-65% with 96-98% purity (scheme-2).

Compound 5 contained five process related impurities 4, 6-9 at the level of 0.1 % when synthesized at a commercial batch level. In order to control all these impurities at this stage, compound 5 was isolated from acetonitrile and all the impurities are controlled up to less than 0.1% in lab as well as plant batches. As we observed that, the acetonitrile used for the isolation of 5 is trapped in Active Pharma Ingredient (API) 1 as residual solvent in 5. Recrystallization of 5 from acetone resolved this problem (Table 2). In reported process, compound 5 was isolated by using column chromatography and recrystallized from a mixture of diethyl ether and n-hexane. We have eliminated this column chromatography unit operation in our process.

Conversion of compound 5 in to Rocuronium bromide 1 by reacting with allyl bromide in dichloromethane is the final stage in the synthetic sequence. Getting the API 1 having a purity meeting the regulatory requirement without using column chromatography is the major challenge in this process. The reaction of 5 and allyl bromide in dichloromethane and catalytic amount of water at 25-30ºC provided the drug substance 1 in 90% yield. Reported reaction time of 22-24 hrs was shortened to 10 hrs by adding catalytically amount of water to the reaction mixture.

Unreacted allyl bromide was removed as azeotropic combination with dichloromethane under vacuum and compound 1 was isolated using an optimized ratio of mixture of dichloromethane and methyl tertiary butyl ether and further purified using the same mixture of solvent to furnish the drug substance 1 with 99.8% purity. Eight impurities 3, 5, 10-15 are well under control (Table-3).

CONCLUSION

We have provided an industrially viable and scalable manufacturing process for Rocuronium bromide having the purity meeting regulatory requirements.

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