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A new synthetic process for tranexamic acid from ethyl 4-oxo-cyclohexane carboxylate

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ABSTRACT

*Synthesis of **trans**-4-aminomethylcyclohexane-1-carboxylic acid (Tranexamic acid) has been achieved by a new synthetic route starting from ethyl 4-oxocyclohexane-1-carboxylate **(1)** using simple and commonly available reagents and under mild conditions. The compound-**1** has been converted into its cyanohydrin **(2)** using sodium cyanide and then, dehydrated with $POCl_3$/ Pyridine to obtain ethyl 4-cyanocyclohex-3-ene-1-carboxylate **(3)**. It has been saponified to acid using methanolic KOH to get corresponding acid, 4-cyano-cyclohex-3-ene-1-carboxylic acid **(4)** which has then been subjected to reductive amination in methanolic ammonium hydroxide over the catalyst Raney nickel to obtain a mixture of 4-aminomethyl-3-cyclohexene-1-carboxylic acid **(5)** and its saturated analogue **(6)**. It has been further subjected to catalytic hydrogenation over wet Pd-C (10%) in aq. methanol to obtain an isomeric mixture of 4-aminomethylcyclohexane-1-carboxylic acids **(7)**. This, on purification by recrystallizatoin from acetone-water (4:3) has yielded pure Tranexamic acid **(6)**. Purity of all intermediates and the title compound has been determined by GC and HPLC analyses and characterized based on their spectral and elemental analyses. Tranexamic acid obtained by this process has been of Pharma grade. Merits of the developed process are presented.*

Keywords: Ethyl 4-oxoxylohexane-1-carboxylate, Ethyl 4-hydroxy-4-cyanocyclohexane-1-carboxylate, Reductive amination, Saponification, Tranexamic acid, haemostatic and antifibronolytic agent.

INTRODUCTION

trans-4-Aminomethylcyclohexane-1-carboxylic acid or Tranexamic acid is known to be a good haemostatic and a proven antifibrinolytic agent. Medically, it is used as a first-time non-harmonal treatment of dysfunctional uterine bleeding associated with uterine fibroids. It is also recommended clinically, for bleeding or risk of bleeding increased upon fibrinolysis, neoplasms, gastrointestinal bleeding, haematuria and post-operative bleeding. Tranexamic acid is proved to be advantageous for causing lesser side effects, in comparsion with the other known fibrinolytic agents.

A survey of literature of Tranexamic acid has revealed the following information on its synthetic processes. The product Patent[1] disclosed a process for 4-aminomethylcyclohexane-1-carboxylic acid from 4-cyanocyclohexane-1-carboxylic acid ester, but claimed using its saponified product, the acid. This was followed by some Process Patents,[2-7] using different starting materials viz: 4-acetamidomethyl-benzoic acid[2] ; 1,4-dimethyl cyclohexane dicarboxylate[3] ; diethyl 4-oxocyclohexane-1,1-dicarboxylate[4] ; 4-cyanobenzoic acid[5], and cyclohexane-1,4-

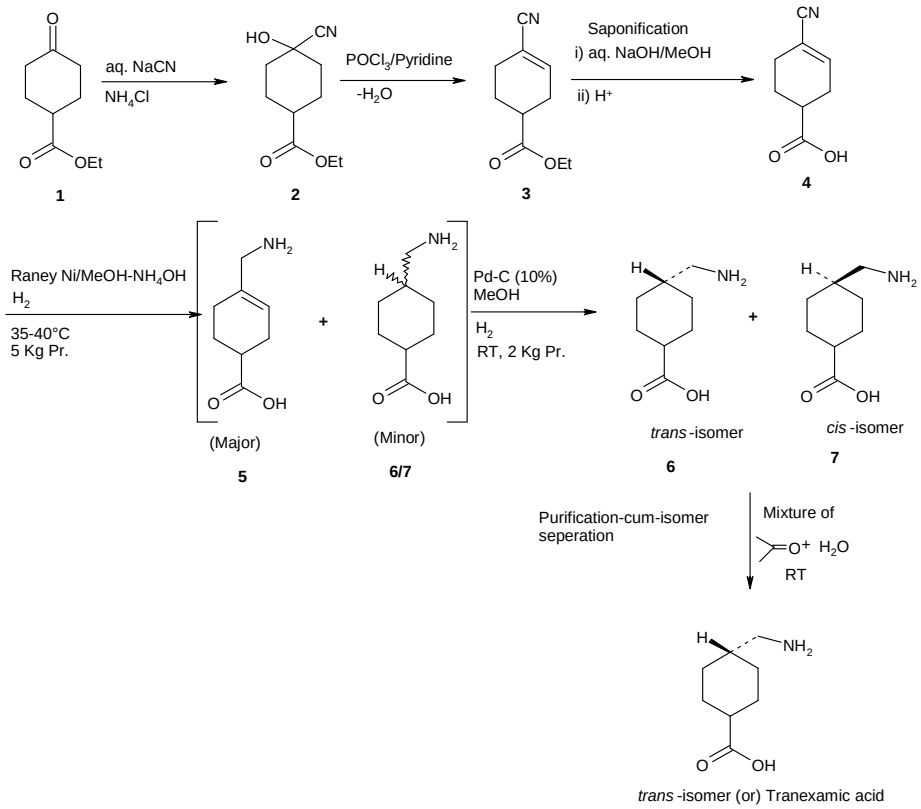
dimethanol[6] ; and it has been also prepared from 4-acetamidomethyl benzoic acid[7] . The conversion of the racemic (***cis***– & ***trans***–) mixture of 4-aminomethylcyclohexane-1-carboxylic acid into the useful ***trans***-isomer by a thermal reaction, in aqueous medium has been disclosed in the US Patent.[8]

Similarly, synthesis of 4-aminocyclohexane-1-carboxylic acid has been also reported in some of the journals, starting from different chemical substrates and they are :

4-methylbenzonitrile[9]; and diethyl4-oxocyclohexane-1.1dicarboxylate[10] which was also covered in one of the US Patent[4], using dimethyl ester of terephthalic acid.[11]

All those processes known so far, for the synthesis Tranexamic acid have disclosed using costly reagents and catalysts along with elevated pressure and temperature which make these process as relatively very costly and difficult to execute. Therefore, in view of greater pharmaceutical and medicinal importance of the Product Tranexamic acid and its commercial demand, it has been considered worthwhile to develop an alternative process starting from readily available raw material and using relatively cheaper reagents, catalysts and experimentally feasible reaction conditions. The results achieved in the investigation are presented in this communication.

The presently developed process for Tranexamic acid, made use of ethyl 4- oxocyclohexane-1-carboxylate as the starting material which was not reported so far, for this purpose. Synthesis of the title compound has been achieved from this new starting material, as presented in the following scheme :



Ethyl 4-oxocyclohexane-1-carboxylate **(1)** has been subjected to nucleophilic reaction involving its oxo-group using aq. sodium cyanide in the presence of ammonium chloride to obtain the respective cyanohydrin (2). The cyanohydrins **(2)** has been heated with phosphorous oxychloride and pyridine, to undergo a dehydration reaction, yielding ethyl 4-cyanocyclohex-3-en-1-carboxylate **(3)**. The ethyl ester (3) has been saponified using aq. sodium hydroxide in methanol to generate the acid **(4)**. The resulted 4-cyanocyclohex-3-en-1-carboxylic acid has been subjected to catalytic hydrogenation over Raney Nickel in methanolic ammonia, at a temperature of about 40°C and 6 Kg pressure to obtain 4-aminomethylcyclohex-3-en-1-carboxylic acid **(5)** as a major product along with a small

amount of 4-aminomethylcyclohexan-1-carboxylic acids **(6 & 7)**. This mixture of acids, on further catalytic hydrogenation over Pd-C (10%) at 5-6 Kg/Cm^2 pressure and ambient temperature lead to the formation of a mixture of ***cis***- and ***trans***- 4-aminomethylcyclohexane-1-carboxylic acid. Its purification by recrystallization from a mixture of acetone-water resulted in separation of a solid product which was proved to be the ***trans***-isomer **(6)** while the ***cis***-isomer **(7)** remained in the filtrate.

All intermediates and the final product have been isolated, purified and characterized and the pertaining data is presented under Experimental Section.

MATERIALS AND METHODS

Melting points of all the compounds, expressed in 0°C were determined using POLMON Melting Point Instrument and are uncorrected. Purity of the compounds was checked by TLC and determined by GC or HPLC analyses. Infra-red spectra of intermediates and final compound were obtained in KBr using Infra-red spectrophotometer and all the characteristic absorption frequencies are expressed in Cm^{-1}, the ^{1}H-NMR and ^{13}C-NMR spectra were taken on 300 MHz NMR spectrophotometer using TMS as an internal standard and all chemical shifts are expressed in Cm^{-1} δ, ppm along with their multiplicity. GC/HPLC, DIP-Mass method has been used to record Molecular ions, and their fragments.

Chemicals & Reagents: The required ethyl 4-oxocyclohexane-1-carboxylate was obtained from commercial source.

Experimental Procedures:

a)Synthesis of Ethyl 4-cyano-4-hydroxycyclohexane-1-carboxylate (2):

A solution of ammonium chloride (40.86 g) in water (650 mL) taken in a RB flask (1.0 L) was cooled to 5-10°C in an ice-bath. Then, added a solution of ethyl 4-oxocyclohexane-1-carboxylate (1; 100 g) in dichloromethane (400 mL), slowly while stirring and not allowing the temperature to rise beyond 2-3°C. Continued to stir for 20-30 minutes after the solution turned turbid. Meanwhile, sodium cyanide (31.65 g) was dissolved in water (100 mL) and added drop-wise to the reaction mixture, at 5-10°C, over a period of 30-45 minutes, when the reaction mixture turned to an off-white turbid solution. Maintained the temperature of this turbid solution at 5-10°C for 2.5 to 3.0 hours, to complete the reaction (TLC). Then, the reaction mixture was extracted with dichloromethane (150 mL) and the left-out aqueous layer was extracted with the same solvent thrice, using 75 mL each time. The combined dichloromethane extracts were dried over anhydrous sodium sulfate and the solvent was removed by distillation at 38±2°C to obtain a pale yellow liquid (~115 g; 97-99%); GC purity : 94-95% A.

IR (in KBr, Cm^{-1}) : 3440 (-OH Str.), 2991, 2954, 2907 & 2872 (C-H aliphatic str.), 2234 (-C≡N str.), 1729 (ester C=O str.) 1456, 1448 (-CH_2- vibr.), 1380,1320 (CH_2 deformation), 1191,1138 (C-N str.), 1093, 1041(C-O str.)

^{1}H NMR (in $CDCl_3$, δ, ppm) : 1.289-1.242 (**t**, 3H, ester CO–CH_2-C**H_3**), 2.47 to 1.62 (**m**, 10H, cyclohexyl 9H + hydroxy O**H**), 4.18-4.11 (**q**, 2H, ester CO–C**H_2**-CH_3).

^{13}C-NMR (in $CDCl_3$, δ, ppm) : 174.44 (**C**=O, ester), 121.61(**C**≡N), 68.72 (**C**-4, cyclohexane), 60.69 (-**C**H_2, ester), 40.84 (**C**-1, cyclohexane), 36.28, 34.98 (**C**-3 & **C**-5, cyclohexane), 24.67 (**C**-2 & **C**-6, cyclohexane), 13.82 (**C**H_3, ester).

Mass (GC-MS – Dip-Mass Method): [M+H]+NH_3 at m/z 215.2.

b)Synthesis of Ethyl 4-cyanocyclohex-3-ene-1-carboxylate (3):

Pyridine (160 mL), taken into a reaction flask was cooled to 0-5°C, in an ice-bath. Then, ethyl 4-cyano-4-hydroxycyclohexane-1-carboxylate (2; 100 g) was introduced slowly, while stirring and maintaining the temperature at 2-6°C. Rinsed the inner walls of the reaction flask with 2-3 mL of pyridine. Phosphorous oxychloride (79 g) was added drop-wise, slowly to the above cooled solution over a period of 60-70 minutes at 0-5°C. When the colourless reaction mixture turned to light pink., stirred the reaction mixture for 30 minutes at 0-5°C. Then, heated to 80°C, in a water-bath and maintained at that temperature for 3 hours to complete the reaction (TLC). The reaction mixture was quenched with crushed ice (~200g) and stirred well. It was stirred with ethyl acetate (100 mL), allowed to settle and separated the organic layer. The left-out aqueous layer was extracted, thrice with ethyl acetate, using 100 mL each time, and combined all the solvent extracts. Washed the solvent extracts with water and then with 10% sodium bicarbonate solution, followed by brine (200 mL), and dried. The solvent was distilled–off under vacuum at 55-60°C to obtain the product (80.5 g; 88.6%) as a grey liquid, with GC purity : 94-95% A

b.p 124-126°C (lit: 125-127°C).[12]

IR (in KBr; in Cm^{-1}) : 2981, 2939, 2874 (aliphatic C-H str.), 2215 (C≡N, str.), 1730 (ester C=O str.), 1641 (C=C str.), 1447 (-CH_2-), 1379, 1330 (-CH_2- deformation), 1226, 1182 (C-N str.) 1039, 1029 (C-O str.).
^{1}H NMR (in $CDCl_3$, δ, ppm) : 1.29 (**t**, 3H, C**H_3**), 2.30-2.29 (**m**, 2H, C_4-**H**, cyclohexane), 2.35-2.34 (**d,** 2H, C_6-**H**, cyclohexane), 2.48-2.44 (**m**, 2H, C_2-**H**, cyclohexane), 2.60-2.54 (**m**, 1H, C_1-**H**, cyclohexane), 4.20 (**q**, 2H, COO-C**H_2**-CH_3), 6.46 (**q**, 1H, C_5-**H**, cyclohexane).
^{13}C-NMR (in $CDCl_3$, δ, ppm) : 174.08 (ester, **C**=O), 143.12 (=**C_3,** cyclohexene),118.82 (≡**C,** nitrile), 111.62 (cyclohexene, =**C**-4), 60.64 (-**C**H_2, ester), 49.85 (**C**-1, cyclohexane), 27.48 (**C**-6, cyclohexane), 25.42 (**C**-2, cyclohexane), 23.67 (**C**-5, cyclohexane), 13.81 (**C**H_3, ester).
Mass (LC-MS, Dip-Mass Method) : M+1 : 180.22

c) Synthesis of 4-Cyanocyclohex-3-en-1-carboxylic acid (4):
A mixture of ethyl 4-cyanocyclohex-3-en-1-carboxylate (3; 45 g) and methanol (125 mL) was taken into a reaction flask, stirred well and cooled the solution to 0-5°C, in an ice-bath. Then, added a cold aqueous solution (125 mL) of potassium hydroxide (15.5 g) to the reaction mixture, slowly, drop-wise at 0-5°C, over a period of 60-70 minutes. Allowed the reaction mixture to reach to 28±2°C and continued to stir for overnight (16-18 hours), while following the TLC for completion of the reaction. Distilled-off methanol at 45±5°C using vacuum and the residue was triturated with water (~100 mL), and extracted the organic matter with Dichloromethane (~100 mL). The aqueous layer was cooled to 0-5°C and acidified to pH 1-2 with hydrochloric acid (~38 mL). Then, extracted with dichloromethane (~100 mL), first, followed by two more successive extractions using Dichloromethane (~75 mL) each time. Combined all the Dichloromethane extracts, washed twice with water (~75 mL), each time and finally with brine (50 mL). The extracts were dried over anhydrous sodium sulfate and distilled at 35±2°C to obtain a solid. It was purified by stirring with di-iso-propylethyl ether (75 mL), for an hour. Filtered washed with cold di-iso-propyl ethyl ether (~5 mL) and dried under vacuum ; 32.5 g (84%).

m.p. 118-120°C HPLC Purity : 98.5-99.0% A

IR (in KBr, Cm^{-1}) : 3395 (**br trough**; -OH str. of CO_2H), 2938, 2910 (C-H str. cyclohexane satd), 2720, 2657 (C-H str. ethylenic), 2216 (C≡N str.), 1697 (C=O str. –CO_2H), 1638 (C=C str. ethylenic), 1459, 1428, (-CH_2- vibr.), 1310, 1267, 1260, 1247, (C-N str.), 1148, 1115, 1073, 1040 (C-O str.).
^{1}H NMR (in $CDCl_3$, δ, ppm) : 10.20 (**bs,** 1H, -CO_2**H**), 6.61-6.64 (**s,** 1H, C_3-**H**, ethylenic), 2.62-2.69 (**m,** 1H, C_1-**H**), 2.48-2.50 (**t,** 2H, C_2-**H**), 2.31-2.40 (**m,** 2H, C_5-**H**), 2.08-2.15 (**m,** 1H, C_5-**H_e**), and 1.77-1.83 (**m,** 1H, C_5-**H_a**).
^{13}C-NMR (in $CDCl_3$, δ, ppm) : 180.18 (-**C**OOH); 142.72 (=**C**-3,cyclohexene); 118.67 (-**C**≡N); 111.17 (=**C**-4, cyclohexene); 36.91 (**C**-1, cyclohexene); 27.14 (**C**-6); 25.26 (**C**-2); 23.37 (**C**-5)
Mass (LC-MS – Dip-Mass Method) : Molecular ion was recorded in Negative Mode M-1 = 150 m/z (Mol. Wt : 151).

d) Synthesis of 4-Aminomethylcyclo hex-3-en-1-carboxylic acid (5):
4-Cyanocyclohex-3-en-1-carboxylic acid (4; 20.0 g) was taken into a reaction flask, dissolved in methanol (200 mL) and added activated carbon (~2.0 g). It was stirred at RT, for 10-15 minutes and filtered-off the carbon. The clean methanolic solution was taken into an autoclave flask and added Raney Nickel (~8.0 g), then ammonium hydroxide solution (~43 mL, 16-17 %). Applied on hydrogen pressure (6-8 Kg/cm^2) and maintained for about 24 hours at RT while maintaining the hydrogen pressure at 6-8 Kg/Cm^2 (It required application of hydrogen, for about 4-5 times) and following the TLC. Transferred the reaction mixture into a beaker, after the reaction was over (TLC), filtered-out the catalyst, Raney Nickel and washed twice with methanol (10 mL, each time). Then, the solvent was distilled-off at 75-80°C, under vacuum, over a period of ~2 hours, to obtain a greenish solid. It was purified by stirring with methanol (70-75 mL), 1-1 ½ hour, at RT, filtered, washed twice with methanol (15 mL) and dried under vacuum to obtain an off-white-faintly green solid ~10.5 g (50±1 %), which was shown to be a mixture of 4-aminomethylcyclohex-3-en-1-carboxylic acid (5) and its saturated analogue (6/7), on HPLC analysis.

IR spectrum (in KBr, Cm^{-1}) exhibited: 3339 & 3274 (-NH_2, str.), confirming the primary amino group resulted from substrate nitrile, on reduction.

This was confirmed by its **mass spectrum** by **LC-MS Dip-Mass method,** which recorded two characteristic M+1 ions at : m/z 157.1 (for saturated) and 155.1 (for unsaturated).

e) Synthesis of *trans*-4-aminomethylcyclohexane-1-carboxylic acid or Tranexamic acid :

The mixture of saturated and unsaturated 4-aminomethylcyclohexane-1-carboxylic acid (6/7; 14.0 g) obtained from previous stage was taken into a reaction flask along with water (~190 mL), added active carbon (~2 g) and stirred at RT, for 15-20 minutes. Carbon was filtered-off, washed with small portions of water and transferred the clear solution into an autoclave flask. Then, introduced methanol (~20 mL) and the catalyst Pd-C (10% ; 1.0 g).The flask was fixed in an autoclave and applied 5-6 Kg/cm^2 hydrogen pressure. It was maintained at that pressure for 18-20 hours, at RT while checking the reaction for completion by TLC. Trans**f**erred the reaction mixture into a beaker, after completion, and filtered carefully to separate the catalyst and washed twice with water (10 mL). Then, removed the water by distillation under vacuum, at 80±2°C over 3 hours to obtain an off-white crude ~15 g (wet). It was dissolved in cold water (40 mL) and cooled further to 0-5°C. Then, added acetone (60 mL), portion-wise, stirred for about 1½ hours and filtered. Washed the product twice with acetone (10 mL, each) and dried. This resulted in a colourless solid, ~7.0 g (~50%) with HPLC purity : 99.75% A.

m.p (°C, DSC) : 262.76 to 303.03°C (decomp.)

IR (in KBr, Cm^{-1}) : 3335 & 3289 (NH_2 str.), 2935, 2865 (CH_2 str. aliphatic), 1647 (C=O str. $–CO_2H$), 1570, 1541 (NH_2 deformation), 1434, 1452 (CH_2 scissoring vibr.), 1382, 1328 (-CH_2- spacing sym. deformation in amino acids), 1279, 1254, 1227 & 1195, 1162 (C-O str. in CO_2H), 1089 (C-N str. aliphatic amines), 1030, 1009, 976, 921 (C-C carbon ring) ring breathing.

^{1}H NMR (in D_2O, δ, ppm) : 2.81-2.84 **(d,** 2H, -C**H**$_2$-N linked to C_4), 2.15-2.04 (**m,** 1H, C_1-**H**), 1.95-1.82 (**dd,** 4H, C_2-**H** & C_6-**H**), 1.42-1.30 (**q,** 2H, C_3-**H**), 1.10-0.99 (**q,** 2H, C_5-**H**), 1.66-1.62 (**br,m,** 1H, -N**H**) or 2.15-2.07 (**t,** 1H, -N**H**)

^{13}C-NMR (in D_2O, δ, ppm) : 188.54 (-**C**O_2H); 49.14 (**C**H_2-NH_2); 47.67 (**C_1**, cyclohexane); 37.76 (**C_4**, cyclohexane); 31.69 (**C_3** & **C_5**, cyclohexane); and 31.59 (**C_2** & **C_6**, cyclohexane).

RESULTS AND DISCUSSION

Though the preparation of ethyl 4-cyano-4-hydroxycyclohexane-1-carboxylate (2) from the starting material ethyl 4-oxocyclohexan-4-one-1-carboxylate (1), was reported through patents[2-7], by two other methods, both of them involved costly reagents and chromatographic methods to isolate the cyanohydrin., viz : one of the methods made use of expensive trimethylsilyl cyanide as the reagent along with zinc iodide, as a catalyst in chloroform for the reaction and cumbersome column chromatography to obtain the cyanohydrin. The second method, used the most poisonous potassium cyanide as the reagent along with sodium bisulfite for the reaction and employed column chromatography to get the cyanohydrin. Whereas, in the present method we employed a relatively less toxic, comparatively less costly sodium cyanide along with commonly available ammonium chloride and the resultant cyanohydrin has been directly and easily isolated from the reaction mixture, without any chromatographic method. Then, the dehydration of the cyanohydrin has been carried out using two alternative reagents : phosphorous oxychloride or thionyl chloride with pyridine, use of the later reagent has been reported but not the first. Hydrolysis of resultant ethyl 4-cyano-3-cyclohexen-1-carboxylate has been carried out, simply using aq. potassium hydroxide in methanol (0.1 N), at RT followed by acidification with hydrochloric acid to obtain the respective 4-cyano-3-cyclohexen-1-carboxylic acid. But, the same reaction, covered in a Japanese Patent : JP 48022445 A, dated : 22.03.1973 made use of 5 N sodium hydroxide, washing the reaction mixture with chloroform and then passing over Amberlite IR-120 (H^+) for isolation of the product, which is quite cumbersome, and time consuming.

In our process, 4-cyano-3-cyclohexen-1-carboxylate has been then subjected to catalytic hydrogenation in ammonium hydroxide over Raney nickel catalyst at RT and 5 Kg/cm^{-1} pressure to get a mixture of 4-aminomethyl-3-cyclohexene-1-carboxylic acid and isomers of 4-aminomethyl cyclohexane-1-carboxylic acid. This on further catalytic hydrogenation using Pd/C (10%) as catalyst at RT and 5 Kg/ cm^{-1} pressure. A simple work-up of the reaction mixture in water, has resulted in the product Tranexamic acid, directly not requiring any Resin.

Thus, the presently developed synthetic route and process for Tranexamic acid is very simple in comparison to those routes reported previously, since it makes use of less costlier and easily available chemicals, reagents and catalysts,

and more favourable reaction conditions. Further, the presently developed synthetic process is validated and scalable to manufacturing level, without any difficulty.

CONCLUSION

The present studies on synthesis of Tranexamic acid proved that the product can be prepared from a new starting material ethyl 4-oxocyclohexane-1-carboxylate involving simple reactions, under simple conditions. Further, this synthetic process avoids all the toxic and costly reagents and high pressure reactions. Thus, the present synthetic process is quite novel and useful for the synthesis of Tranexamic acid in laboratory and equally suitable for manufacturing, in industry.

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