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Novel Route of Synthesis of Some Trientine Impurities and Their Characterization

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ABSTRACT

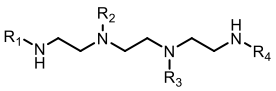
Trientine has been tested for inhibition of the spontaneous development of hepatitis and hepatic tumours in rats. It has been described in the use of copper binding compounds in the treatment of various disorders, including treatment of diabetes mellitus and complications thereof, including diabetic cardiomyopathy. We hereby wish to report first synthesis of Impurities A, B, C, D, E, G and H along with Impurity F via innovative approach starting from cheap raw materials.

Keywords: Trientine, Synthesis, Impurities, Wilson's Disease, Process

INTRODUCTION

Polyethylene polyamines include triethylenetetramines that act as copper antagonists [1]. Triethylenetetramine (TETA), sometimes also referred to as Trientine, is used as a copper chelating agent [2]. The reactivity and uses of TETA are similar to those for the related polyamines ethylenediamine and diethylenetriamine. It is primarily used as a cross linker ("hardener") in epoxy curing [2-4]. It has also been used as a thermosetting resin, as a lubricating oil additive, and as an analytical reagent for copper and nickel [5]. Triethylenetetraminedihydrochloride has also been used in the treatment of patients with Wilson's disease, a rare autosomal recessive inherited disorder of copper metabolism that is characterized by excessive deposition in liver, brain and other vital organs, particularly those are intolerant of penicillamine [6-8]. It has also been reported to treat individuals with primary biliary cirrhosis [9]. In addition, Trientine has been tested for inhibition of the spontaneous development of hepatitis and hepatic tumours in rats [10]. Trientine has been described in the use to substantially reduce the symptoms of diabetes its sequelae like renal dysfunction, visual dysfunction, cardiovascular disease, wound healing problems, etc [11].

The safety of a drug is not only dependent on the toxicological properties of the bulk drug itself but also on the impurities in the bulk drug, and the presence of these unwanted impurities, even in small amounts, may influence the efficacy and safety of the pharmaceutical product [12,13]. Therefore, identification, quantification, and control of impurities in the drug substance and drug product are important steps in drug development and regulatory assessment [14,15]. In our pursuit of developing a convenient and economically viable process for obtaining different impurities (Figure 1) related to Trientine conforming to regulatory specifications, we carried out extensive experimentation aimed at synthesizing and isolating handful quantity.



Trientine: R_1=R_2=R_3=R_4=H
Imp **A** (**1**): R_1=R_3=PhCO, R_2=R_4=H
Imp **B** (**2**): R_1=R_4=PhCO, R_2=R_3=H
Imp **C** (**3**): R_1=R_2=R_3=PhCO, R_4=H
Imp **D** (**4**), R_1=R_2=R_4=PhCO, R_3=H
Imp **E** (**5**): R_1=R_2=R_3=R_4=PhCO
Imp **F** (**6**): R_1=PhCO, R_2=R_3=R_4=H
Imp **G** (**7**): R_1=R_4=H, R_2=R_3=PhCO
Imp **H** (**8**): R_1=R_2=PhCO, R_3=R_4=H

Figure 1: Structures of Trientine and its impurities

At the onset of our journey we anticipated that we could use Trientine free base as our starting raw material for the synthesis of all the impurities. Thorough literature search revealed that there were no reports of synthesis of impurities **A, B, C, D, E, G** and **H.** However, we could find a literature precedence [16] for the synthesis of impurity **F** (6) **(Figure 1)** amid a mixture which was not presented in purified form. However, this method cannot be used for the synthesis of all the impurities. Moreover, the setup for the synthesis of impurity F is challenging and the process seems to be far from practicality. Apart from this, there are reports [17,18] for the synthesis of a bis-salicylate derivative of Trientine, similar to impurity B (2), using Trientine and double equivalents of ethyl salicylate under neat condition. However, there is no synthesis reported for impurity **B**. Therefore, it is an utmost need to have a robust, reproducible and practically viable process to synthesize all the impurities in good yields. We hereby wish to report first synthesis of impurities **A, B, C, D, E, G** and **H** along with new synthetic route for the synthesis of impurity **F** (6) following systematic and practically viable process starting from cheap and widely available raw materials.

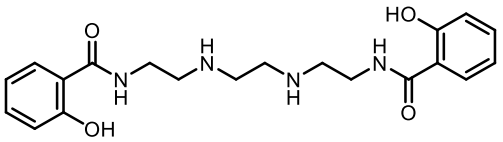


Figure 2: Structures of bis-salicylate derivative of Trientine

EXPERIMENTAL

Unless otherwise indicated, all reactions were carried out with magnetic stirring and in case of air- or moisture-sensitive compounds reactions were carried out in oven-dried glassware under nitrogen. Syringes were used to transfer the reagents and the solvents were purged with nitrogen prior to use. Reactions were monitored by thin-layer chromatography (TLC) analysis [19] using aluminium sheets silica gel 60 F_{254} TLC plates. Column chromatography was carried out using silica gel (100-200 mesh) unless stated otherwise. All the reaction solvents were LR grade. The yields reported are not optimized. Mostly the intermediates were not purified conventionally but were filtered through silica bed making a slury in appropriate solvent system and were not confirmed through spectroscopic techniques. All final compounds were determined to be at least >90% pure by HPLC (high performance liquid chromatography) and ^{1}H NMR spectroscopy. The ^{1}H NMR spectra were recorded with a Bruker 600 MHz spectrometer and the ^{13}C NMR spectra were recorded with a Bruker 150 MHz spectrometer in different deuterated solvents and the chemical shifts are reported in ppm downfield to TMS ($\delta = 0$) for ^{1}H NMR and ^{13}C NMR. The IR spectra were recorded using Perkin Elmer – Spectrum 1 or 100 FTIR. The electron ionization mass spectra were recorded using Waters QDA Aquity – ESI (eV 10 or 15).

Synthesis of Impurity A (1)

To a cooled (0 ºC) stirred solution of ethylenediamine (20 g, 22.25 mL, 332.8 mmol) and potassium carbonate (9.99 g, 166.5 mmol) in acetonitrile (2 L), a solution of benzoic anhydride (37.6 g, 166.2 mmol) in acetonitrile (600 mL) was slowly added drop wise. The reaction mixture was stirred for 7 h at the same temperature. After completion of reaction the solids were filtered and washed with dichloromethane. The mother liquor was distilled under reduced pressure to a residue which was filtered through a column using 20% methanol in dichloromethane to afford 24 g of compound **9**.

To a cooled (10 ºC) solution of compound **9** (5.4 g, 32.9 mmol) in acetonitrile (120 mL) thriethylamine (4.33 g, 5.96 mL, 42.79 mmol) was added followed by the addition of 2-chloroacetonitrile (2.73 g, 2.29 mL, 36.16 mmol). The reaction mixture was stirred at room temperature for 10 h. The solvents were distilled off under reduced pressure and the residue was dissolved with dichloromethane (150 mL). The organic layer was washed with water (2x50 mL) and was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **10**. The residue was dissolved in ethyl acetate and filtered through a bed of silica to afford (4.0 g) sufficiently pure compound **10** which was dissolved in dichloromethane (120 mL), cooled to 10 ºC and triethylamine (2.588 g, 3.56 mL, 25.58 mmol) was added followed by the addition of di-*tert*-butyl dicarbonate (4.296 g, 4.57 mL, 19.68 mmol). The reaction mixture was stirred at room temperature for 4 h. The reaction mixture was cooled to 10 ºC. Another lot of di-*tert*-butyl dicarbonate (0.86 g, 0.915 mL, 3.94 mmol) was added and the reaction mixture was stirred at room temperature for further 2 h. The reaction mixture was diluted with dichloromethane (400 mL) and washed with water (2x60 mL). The organic layer was over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to yield crude compound **11**. The crude mass was dissolved in 30% ethyl acetate in hexane and filtered through a bed of silica to afford (5.1 g) sufficiently pure compound **11**.

To a cooled (10 ºC) solution of compound **11** (5.0 g, 16.48 mmol) in methanol (140 mL) nickel(II) chloride hexahydrate (0.32 g, 1.35 mmol) was added followed by the addition of sodium borohydride (1.292 g, 34.15 mmol). The reaction mixture was stirred at room temperature till the disappearance of compound **11**. The reaction mixture was quenched with water and methanol was removed under reduced pressure. The mass was diluted with water (50 mL) and extracted with dichloromethane (2x50 mL). The organic layer was washed with water; dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to yield crude intermediate **12**. The crude mass was taken in 10% methanol in dichloromethane and filtered through a bed of silica to afford (3.9 g) of compound **12**.

To a cooled (10 ºC) solution of compound **12** in acetonitrile (95 mL) triethylamine (1.54 g, 2.12 mL, 15.22 mmol) was added followed by the addition of 2-chloroacetonitrile (1.05 g, 0.88 mL, 13.9 mmol). The reaction mixture was stirred at room temperature for 10 h. The solvents were distilled under reduced pressure and the mass was diluted with dichloromethane (50 mL). The organic layer was washed with water (2x25 mL), dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **13**. The crude mass was taken in ethyl acetate and filtered through a bed of silica to afford (3.9 g) of compound **13 (**4.4 g).

To a cooled (10 ºC) solution of compound **13** (400 mg, 1.15 mmol) in dichloromethane (20 mL) triethylamine (152 mg, 209 mL, 1.502 mmol) was added followed by drop wise addition of benzoyl chloride (178 mg, 147 mL, 1.266 mmol). The reaction mixture was stirred at the same temperature till disappearance of compound **13**. The reaction mixture was diluted with dichloromethane (40 mL) and the organic layer was successively washed with water (40 mL), saturated sodium bicarbonate solution (20 mL) and water (20 mL). The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **14**. The crude mass was taken in 50% ethyl acetate in hexane and filtered through a bed of silica to afford compound **14 (**0.46 g) leaving behind maximum unwanted materials.

To a cooled (-5 ºC) solution of compound **14** (450 mg, 0.99 mmol) in methanol (22 mL) di-*tert*-butyl dicarbonate (432 mg, 0.45 mL, 1.98 mmol) was added followed by slow addition of nickel(II) chloride hexahydrate (26 mg, 0.2 mmol) and sodium borohydride (261 mg, 6.9 mmol). The reaction mixture was stirred at 0 ºC for 1 h. The reaction mixture was quenched with water (7 mL) and methanol was removed under reduced pressure. The mass was diluted with water (33 mL) and extracted with ethyl acetate (2x35 mL). The organic layer was washed with water; dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure to yield crude **15** (450 mg).

This crude compound **15** (450 mg, 0.811 mmol) was dissolved in acetone (6 mL) and concentrated hydrochloric acid (112 mL) was added at room temperature. The reaction mixture was heated to 50 ºC till the disappearance of the starting material. The reaction mass was cooled to room temperature and the solvents were decanted leaving behind gummy mass. The mixture was triturated with *n*-hexane to obtain impurity **A**(**1**) as hygroscopic solid (210 mg, 66.2%, HPLC purity 93.7%).

IR (KBr): 3417, 2967.3 and 1633.5 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): d_H 9.26 (brs, 1H), 8.91 (brs, 1H), 8.16-8.13 (m, 2H), 7.96-7.92 (m, 2H), 7.55-7.51 (m, 2H), 7.48-7.40 (m, 4H), 3.82-3.71 (m, 2H), 3.70-3.50 (m, 3H), 3.50-3.38 (m, 5H), 3.27-2.96 (m, 5H). ^{13}C NMR (150 MHz, DMSO-d_6): d_C171.89, 166.67, 135.79, 133.78, 131.40, 129.51, 128.36, 128.21, 127.41, 126.78, 48.60, 46.60, 40.00 and 35.80. M/z (EIMS): 355.3 (M+1).

Synthesis of Impurity B (2)

To a stirred solution of ethylenediamine (761 mg, 0.85 mL, 12.66 mmol) in acetonitrile (10 mL) potassium carbonate (3.66 g, 26.48 mmol) was added and the reaction mixture was cooled to 10 ºC. 2-Chloroacetonitrile (2.0 g, 1.68 mL, 26.49 mmol) was added and the reaction mixture was stirred at room temperature till the disappearance of ethylenediamine. The reaction mixture was again cooled to 10 ºC anddi-*tert*-butyl dicarbonate (5.78 g, 6.08 mL, 26.48mmol) was added slowly. The reaction was continued for 6 h at room temperature. The reaction mixture was diluted with ethyl acetate (60 mL) and washed successively with water (30 mL) and brine solution (10 mL). The separated organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **16** which was purified through bed of silica using 25% ethyl acetate in hexane to afford 3.2 g (74.7%) of compound **16**.

To a suspension of Raney Ni (1.8 g) in 2-propanol (8 mL) was added ammonia in 2-propanol (8.9%, 30 mL) followed by the addition of compound **16** (3.0 g, 8.87 mmol). Then hydrogen gas was applied with 2.5 kg/cm^2 pressure. The reaction mixture was stirred at room temperature under this condition for 16 h. The reaction mass was filtered through hyflow and washed the bed with 2-propanol (50 mL). The filtrate was evaporated to dryness under reduced pressure to provide crude compound **17** which was purified through bed of silica using 5% methanol in dichloromethane to afford 1.1 g, (35.8%) of pure compound **17**.

To a stirred solution of compound **17** (0.3 g, 0.866 mmol) in dichloromethane (5 mL) triethylamine (0.2 g, 0.28 mL, 1.98 mmol) was added followed by the addition of benzoic anhydride (400 mg, 334 mL1.77 mmol). The reaction mixture was stirred at room temperature for 2 h. The reaction mixture was diluted with dichloromethane (30 mL). The organic layer was washed with water (20 mL) and dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **18**.The crude mass was taken in 80% ethyl acetate in hexane and filtered through a bed of silica to afford compound **18 (**0.42 g).

To a cooled (10 ºC) solution of compound **18** (280 mg, 0.504 mmol) in dichloromethane (5 mL) trifluoroacetic acid (250 mg, 168 mL, 2.22 mmol) was added and the reaction mixture was stirred for 3 h at 10 ºC. The reaction mixture was distilled off under reduced pressure and the residue was taken in dichloromethane (5 mL) and washed with saturated sodium bicarbonate solution. The organic layer was dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure to afford crude impurity **B**which was purified through a column using 10% methanol in dichloromethane to afford impurity **B** (**2**) (0.14 g, 78.3%, HPLC purity 97.3%).

IR (KBr): 3074.3, 2930.2, 1661.9, 1208.8, 1171.3 and 1139.6 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): d_H 8.73 (brs, 2H), 7.87 (d, J = 7.2 Hz, 4H), 7.55 (t, J = 6.9 Hz, 2H), 7.48 (t, J = 7.2 Hz, 4H), 3.57-3.56 (m, 6H), 3.29 (brs, 4H), 3.17 (brs, 4H). ^{13}C NMR (150 MHz, DMSO-d_6): d_C167.10(2C), 133.87 (2C), 131.58 (2C), 128.36 (4C), 127.36 (4C), 46.90 (2C), 43.03 (2C), 36.11 (2C).M/z (EIMS): 355.3 (M+1).

Synthesis of Impurity C (3)

To a cooled (10 ºC) solution of compound **10** (3.0 g, 14.76 mmol) in dichloromethane (20 mL) thriethylamine (1.94 g, 2.67 mL, 19.17 mmol) was added followed by the addition of benzoyl chloride (2.3 g, 1.9 mL, 16.36 mmol). The reaction mixture was stirred at room temperature till the disappearance of compound **10**. The reaction mixture was diluted with dichloromethane (60 mL) and washed successively with water (20 mL) and saturated aqueous sodium bicarbonate solution (20 mL). The separated organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **19** which was filtered through bed of silica using 30% ethyl acetate in hexane to afford intermediate compound **19** (3.0 g).

To a cooled (0 ºC) solution of compound **19** (1.5 g, 4.88 mmol) in methanol (36 mL) di-*tert*-butyl dicarbonate (2.13 g, 2.24 mL, 9.76 mmol) was added followed by the addition of nickel(II) chloride hexahydrate (126 mg, 0.53 mmol) and sodium borohydride (1.29 g, 34.1 mmol). The reaction mixture was stirred at 0 ºC till the disappearance of compound **19**. The reaction mixture was quenched with water and methanol was removed under reduced pressure. The mass was diluted with water (40 mL) and extracted with ethyl acetate (2x40 mL). The combined organic layer was washed with water; dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to yield intermediate **20** (1.5 g). Concentrated hydrochloric acid (0.33 mL) was added to compound **20** (1.5 g, 3.645 mmol) in acetone (15 mL) at room temperature and the reaction mixture was heated to 50 ºC till the disappearance of the starting material. The reaction mass was cooled to room temperature and hexane (5 mL) was added to obtain gummy mass. The solvent was removed and acetone (5 mL) was added followed by *n*-hexane (20 mL). The mass was stirred for 30 min. The solids were filtered and washed with *n*-hexane to provide compound **21** (1.0 g). Triethylamine (0.785 g, 1.08 mL, 7.76 mmol) was added to compound **21** (0.9 g, 2.59 mmol) in acetonitrile (20 mL) and the reaction mixture was cooled to 10 ºC. 2-Chloroacetonitrile (0.215 g, 181 mL, 2.85mmol) was added and the reaction mixture was stirred at room temperature till the disappearance of compound **21**. The reaction mixture was diluted with ethyl acetate (30 mL) and washed successively with water (20 mL) and brine solution (10 mL). The separated organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide a solid mass which was filtered through a bed of silica

using 80% ethyl acetate in hexane to afford pure compound **22 (**0.8 g).

To a cooled (10 ºC) solution of compound **22** (200 mg, 0.57 mmol) in dichloromethane (2 mL) triethylamine (69 mg, 95 mL, 0.682 mmol) was added followed by drop wise addition of benzoyl chloride (93 mg, 77 mL, 0.66 mmol). The reaction mixture was stirred for 2.5 h at room temperature. Methanol (3 mL) was added to the reaction mixture and stirred for further 15 min. Then 10% hydrochloric acid solution (3 mL) was added and stirred for another 30 min. The organic layer was separated, washed successively with water and saturated aqueous sodium bicarbonate solution, dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure to provide a rsidue which was taken in ethyl acetate and passed through a bed of silica to obtain compound **23** (0.255 g). To an ice cooled solution of compound **23** (250 mg, 0.55 mmol) in methanol (4.5 mL) di-*tert*-butyl dicarbonate (0.24 g, 252 mL, 1.1 mmol) was added followed by the addition of nickel(II) chloride hexahydrate (13.1 mg, 0.055 mmol) and sodium borohydride (146 mg, 3.86 mmol). The reaction mixture was stirred at 0 ºC for 1 h. The reaction mixture was quenched with water (3 mL) and methanol was removed under reduced pressure. The mass was diluted with water and extracted twice with ethyl acetate (2x5 mL). The combined organic layer was washed successively with saturated aqueous sodium bicarbonate solution and water; dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure. The residue was dissolved in dichloromethane (3.5 mL) and cooled the solution to 0 ºC.Trifluoro acetic acid (2.05 g, 1.37 mL, 17.98 mmol) was slowly added to the cooled reaction mixture and stirred at room temperature for 2 h. The reaction mixture was distilled off under reduced pressure and the residue was taken in cold saturated aqueous sodium bicarbonate solution. The aqueous layer was extracted with ethyl acetate and the aqueous layer was evaporated to dryness under reduced pressure. The residue was stirred with acetonitrile-methanol (5:3, 8 mL) while solids separated. The solids were filtered off and the mother liquor was evaporated under reduced pressure to dryness to afford sufficiently pure (HPLC purity 92.9%) impurity **C** (0.14 g, 55.5%).

IR (KBr): 3447.9 and 1629.8 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): d_H 8.76-8.66 (m, 1H), 8.50-8.35 (m, 2H), 7.90-7.80 (m, 2H), 7.60-7.09 (m, 13H), 3.81-3.10 (m, 11H), 2.90-2.86 (m, 1H). ^{13}C NMR (150 MHz, DMSO-d_6): d_C171.23, 166.41, 166.04, 136.24, 135.72, 134.37, 131.08, 129.22, 129.06, 128.18 (4C), 127.16 (5C), 126.61, 126.23 (2C), 47.60, 47.25, 46.88, 43.43, 37.41 and 36.62. M/z (EIMS): 459.2 (M+1), 481.24 (M+Na).

Synthesis of Impurity D (4)

To an ice cooled solution of compound **14** (1.8 g, 4.0 mmol) in methanol (45 mL) nickel(II) chloride hexahydrate (94 mg, 0.4 mmol) was added followed by slow addition of sodium borohydride (1.06 g, 28.02 mmol). The reaction mixture was stirred at room temperature for 1 h. The reaction mixture was quenched with water (1 mL) and the solvents were removed under reduced pressure. The residue was taken in minimum quantity of % methanol in dichloromethane was filtered through a bed of silica to afford intermediate **24** (0.91 g). To an ice cooled solution of compound **24** (200 mg, 0.44 mmol) in dichloromethane (2 mL) triethylamine (51 mg, 70 mL, 0.5 mmol) was added followed by drop wise addition of benzoyl chloride (62 mg, 51 mL, 0.44 mmol). The reaction mixture was stirred for 30 min at the same temperature. Methanol (0.5 mL) was added to the reaction mixture and stirred for further 15 min. Saturated sodium bicarbonate solution was added and the mixture was extracted twice with dichloromethane (2x5 mL). The combined organic layer was washed with water, dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure to provide crude compound **25**. The residue was chased twice with toluene (2x5 mL) to afford intermediate **25** (0.245 g). To an ice cooled solution of compound **25** (200 mg, 0.358 mmol) in dichloromethane (2.5 mL) trifluoro acetic acid (1.48 g, 1.0 mL, 12.93 mmol) was slowly added and stirred for 4 h at room temperature. The reaction mixture was distilled off under reduced pressure and the residue was taken in dichloromethane (5 mL) and washed with saturated sodium bicarbonate solution. The organic layer was dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure to afford sufficiently pure (HPLC purity 92.2%) impurity **D** (**4**) (0.15 g, 91.4%).

IR (KBr): 3314.2, 2923.9 and 1637.6 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): d_H 8.73 (brs, 1H), 8.68 (brs, 1H), 7.90-7.77 (m, 4H), 7.52-7.32 (m, 11H), 3.77-3.32 (m, 11H), 2.90-2.70 (m, 2H). ^{13}C NMR (150 MHz, DMSO-d_6): d_C171.72, 166.84, 166.22, 131.38, 131.24, 129.17, 128.24 (6C), 128.14, 127.31 (2C), 127.14 (4C), 126.73, 126.38, 48.48, 47.23, 40.03 (2C), 37.47 and 28.98. M/z (EIMS): 459.24 (M+1).

Synthesis of Impurity E (5)

To a cooled (10 ºC) solution of Trientinedihydrochloride (0.5 g, 2.28 mmol) in acetonitrile (10 mL) 10% solution of sodium hydroxide (0.638 g, 15.95 mmol) was added followed by the addition of benzoyl chloride (1.441 g, 1.19 mL, 10.23 mmol). The reaction mixture was stirred at room temperature for 14 h. The reaction mixture was diluted with ethyl acetate (50 mL) and washed with water (30 mL). The separated organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide crude compound **E**. The crude mass was triturated with methanol (10 mL) to afford impurity **E** (1.1 g, 86%, HPLC purity 99.25%).

IR (KBr): 1655.2 and 1622.1 cm^{-1}. ^1H NMR (600 MHz, CF_3COOD): d_H 8.98 (d, *J* = 12.0 Hz, 1H), 8.91 (d, *J* = 10.0 Hz, 1H), 8.83 (d, *J* = 12.0 Hz, 4H), 8.78-8.73 (m, 2H), 8.71-8.61 (m, 7H), 8.56-8.48 (m, 3H), 8.45 & 8.37 (2d, *J* = 10.0 Hz, 2H), 5.54-5.44 (m, 2H), 5.27-5.14 (m, 4H), 5.12-4.97 (m, 6H), 4.84-4.75 (m, 2H). ^{13}C NMR (150 MHz, CF_3COOD): d_C 179.9, 179.85, 176.98, 176.62, 137.89, 137.72, 135.09, 134.85, 132.22 (2C), 132.17 (3C), 132.10, 132.02 (2C), 131.93 (2C), 130.20, 130.10 (4C), 130.00, 129.23 (2C), 129.14, 128.92. 52.04, 50.54, 48.01, 47.81, 42.46 and 41.57. M/z (EIMS): 563 (M+1), 561 (M-1).

Synthesis of Impurity F (6)

To a cooled (10 ºC) stirred solution of Trientinedihydrochloride (1.0 g, 4.56 mmol) in methanol (40 mL) 10% solution of sodium hydroxide (183 mg, 4.57 mmol) was added followed by a slow drop wise addition of a solution of benzoic anhydride (258 mg, 1.14 mmol) in methanol (20 mL) at 10 ºC over a period of 1 h. The reaction mixture was stirred for 17 h at the same temperature. The solvents were distilled off under reduced pressure to afford compound **26**. This was taken in dichloromethane (10 mL), cooled to 10 ºC and triethylamine (1.214 g, 1.67 mL, 12.0 mmol) was added followed by drop wise addition of di-*tert*-butyl dicarbonate (2.16 g, 2.28 mL, 9.9 mmol). The reaction mixture was stirred for 6 h at room temperature. The reaction mixture was diluted with dichloromethane (40 mL) and washed with water (20 mL). The separated organic layer was dried over anhydrous sodium sulfate and evaporated to dryness under reduced pressure to provide a residue which was taken in 35% ethyl acetate in hexane and filtered through a bed of silica to afford compound **27** (0.45 g). This compound **27** (400 mg, 0.726 mmol) was dissolved in acetone (10 mL) and concentrated hydrochloric acid (132 mL) was added at room temperature. The reaction mixture was stirred at room temperature for 2 h. The solvents were removed under reduced pressure. The residue was stirred in methanol (4 mL) at room temperature for 15 min to obtain solids of impurity **F** (**6) (**200 mg, 96%).

IR (KBr): 3299.5, 2943.4, 1647.1 and 1538.9 cm^{-1}. ^1H NMR (600 MHz, D_2O): d_H 7.84 (d, *J* = 7.8 Hz, 2H), 7.66 (t, *J* = 7.5 Hz, 1H), 7.57 (t, *J* = 7.5 Hz, 2H), 3.80 (t, *J* = 5.4 Hz, 2H), 3.58 (s, 4H), 3.53 (t, *J* = 6.9 Hz, 2H), 3.46-3.42 (m, 4H). ^{13}C NMR (150 MHz, D_2O): d_C174.75, 135.69, 135.55, 131.82 (2C), 130.21 (2C), 51.25, 47.58, 46.48, 46.31, 39.34 and 38.45. M/z (EIMS): 251.20 (M+1).

Synthesis of Impurity G (7)

Phthalic anhydride (4.05 g, 27.34 mmol) was added to a suspension of Trientine free base (2.0 g, 13.68 mmol) in water (30 mL). The mixture was stirred at 100 °C for 1 h. The solvents were distilled off under reduced pressure and acetone (30 mL) was added to the resulting residue and stirred for 15 min. The insoluble white solids were removed by filtration, washed with acetone and sucked dried to give a known[21]bisphthalimido compound **28** (4.01 g). To a cooled (10 °C) suspension of above compound **28** (4.0 g, 9.84 mmol) in dichloromethane (40 mL) triethylamine (2.3 g, 3.17 mL, 22.7 mmol) was added followed by drop wise addition of benzoyl chloride (2.81 g, 2.32 mL, 20 mmol). The reaction mixture was stirred for 2 h at room temperature. Ice-cooled water (100 mL) was added to the reaction mixture and stirred for 1 h. The mixture was extracted with dichloromethane (2x60 mL). The combined organic layer was washed with water, dried over anhydrous sodium sulfate and evaporated to a residue which was taken in 70% ethyl acetate in hexane and filtered through a bed of silica to afford compound **29** (5.1 g).

Hydrazine monohydrate (342 mg, 6.83 mmol) was added to the compound**29** (1.4 g, 2.278 mmol) in methanol (40 mL) and the mixture was refluxed for 10 h. After cooling, the reaction mixture was filtered and the solids were discarded. The mother liquor was evaporated to dryness and purified through a column using 30% methanol in dichloromethane to afford impurity **G** (**7**) (0.7 g, 86.7%, HPLC purity 90.7%).

IR (KBr): 3313, 3270, 2913, 1640, 1198 and 1117 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): d_H 8.42 (brs, 2H), 7.84 and 7.83 (2s, 4H), 7.52-7.50 (m, 3H), 7.46-7.43 (m, 3H), 3.34 (brs, 6H), 2.70-2.68 (m, 5H), 2.67-2.62 (m, 3H). ^{13}C NMR (150 MHz, DMSO-d_6): d_C166.26(2C), 134.58 (2C), 131.02 (2C), 128.20 (4C), 127.14 (4C), 48.60 (2C), 48.46 (2C), 40.03 (2C).M/z (EIMS): 355.0 (M+1).

Synthesis of Impurity H (8)

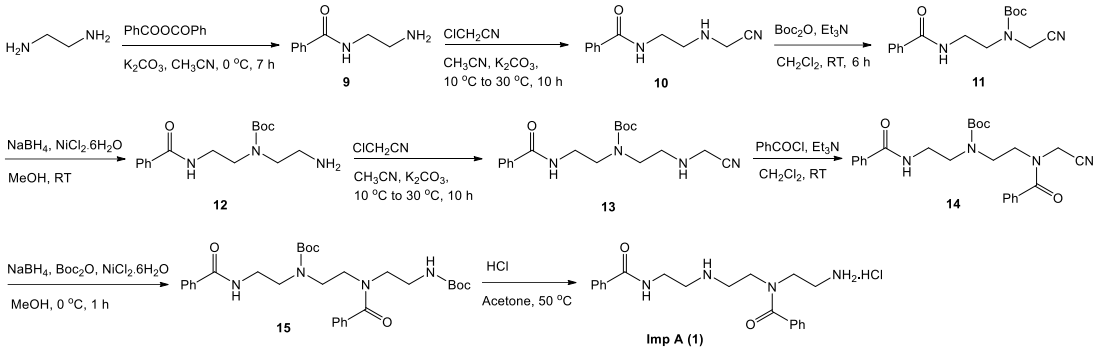
To an ice cooled solution of compound **22** (0.45 g, 1.284 mmol) in methanol (10 mL) di-*tert*-butyl dicarbonate (561 mg, 0.59 mL, 2.57 mmol) was added followed by the addition of nickel(II) chloride hexahydrate (34 mg, 0.144 mmol) and sodium borohydride (116 mg, 3.07 mmol). The reaction mixture was stirred at 10 °C for 1 h and was quenched with water (2 mL). Methanol was removed under reduced pressure. The mass was diluted with water (40 mL) and extracted with ethyl acetate (2x25 mL). The combined organic layer was washed with water; dried (anhydrous Na_2SO_4) and evaporated to dryness under reduced pressure to yield compound **30** (580 mg).

The compound **30** (400 mg, 0.72 mmol) was dissolved in acetone (3 mL) and concentrated hydrochloric acid (0.25 mL) was added at room temperature. The reaction mixture was heated to 50 °C till the disappearance of the starting material **30**. Cooled the reaction mixture to room temperature and the solvents were removed. Acetone (2 mL) was added and the mixture was shaken well when a gummy mass obtained. Acetone was decanted off. Dichloromethane (2 mL) was added to make a clear solution and hexane (10 mL) was added to crystallize the product but again obtained a gummy mass. The gummy mass was chased with toluene twice under reduced pressure to provide impurity **H** (**8**) (161 mg, 57%, HPLC purity 82.6%).

IR (KBr): 3433.8 and 1630.3 cm^{-1}. ^1H NMR (600 MHz, DMSO-d_6): d_H 9.64 (brs, 2H), 8.59 & 8.43 (2brs, 2H), 7.79 (d, *J* = 5.4 Hz, 1H), 7.54-7.28 (m, 10H), 3.83 (brs, 2H), 3.61-3.11 (m, 10H). ^{13}C NMR (150 MHz, DMSO-d_6): d_C171.84, 166.15, 136.17, 133.9, 131.22, 129.15, 128.20 (2C), 127.96, 127.41, 127.17 (3C), 126.91, 48.70, 44.78, 44.45, 41.49, 37.38 and 35.22. M/z (EIMS): 354.9 (M+1).

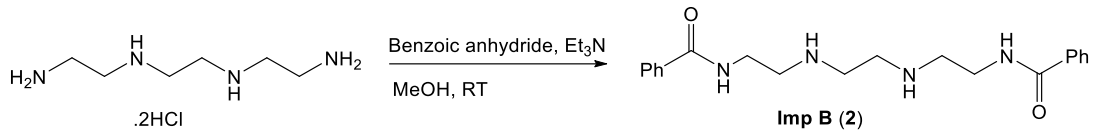
RESULTS AND DISCUSSION

We intended to start our journey with the synthesis of Impurity **A** in eight steps (Scheme 1). This task was advantageous and noteworthy in a sense that we could use some of the intermediates of this scheme for the preparation of other impurities as well. Ethylenediamine was selectively benzoylated using a modified procedure [20] to obtain a known compound **9** [21], which on alkylation [22] with chloroacetonitrile yielded **10**. The secondary amino group of compound **10** was then protected with *tert*-butoxycarbonyl (Boc) to afford compound **11** using a modified and milder condition of Ragnarsson and Grehn method [23] where we circumvented the use of DMAP (4-*N*,*N*-dimethylaminopyridine) to avoid Boc protection on amide nitrogen as well. Our initial attempt to reduce the nitrile group of compound **11** using Raney-Ni following a published procedure [22] furnished poor result. We therefore chose to use a modified method of Caddick and co-workers [24] where we successfully avoided the addition of di-*tert*-butyl dicarbonate to obtain free amine which was suitable for chain elongation. Thus, the obtained compound **12** was alkylated [22] with 2-chloroacetonitrile to afford compound **13**. The intermediate compound **13** was then benzoylated following a reported procedure [25,26] to obtain compound **14** which on reduction [24] with $NaBH_4$ in presence of di-*tert*-butyl dicarbonate afforded **15**. When we treated compound **15** with trifluoroacetic acid (TFA) [27], to obtain impurity **A** although amidst a lot of other impurities. The purification on silica gel using dichloromethane-methanol gave some amount of pure impurity **A**. However, on storing overnight at 2-8 °C it was found to be degraded. The reason was not investigated further but we assumed that the presence of traces of methanol (used in the chromatography) along with silicic acid generated due to use of methanol might have played a role in this decomposition. Therefore we used aqueous hydrochloric acid [28] in acetone at 50 °C to remove the temporary protection. This condition worked well in our hands to afford the impurity **A** (Scheme 1).

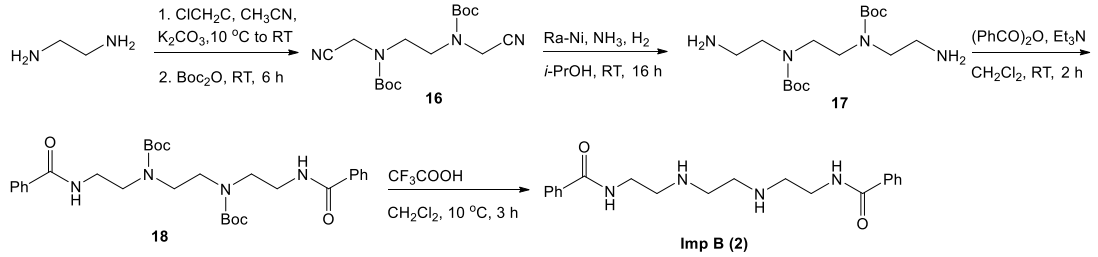


Scheme 1: Synthesis of impurity A

Next, we confined our effort to synthesize impurity **B**. We decided to use Trientinedihydrochloride itself to convert to impurity **B** in a single step following a method published by Li and co-workers [29] using benzoic anhydride in methanol as shown in Scheme 2. However, this method was non-selective and we encountered poor yield of impurity **B** (**2**) in the mixture of other compounds (by HPLC). We were also unable to isolate pure impurity **B** (**2**) from the mixture.



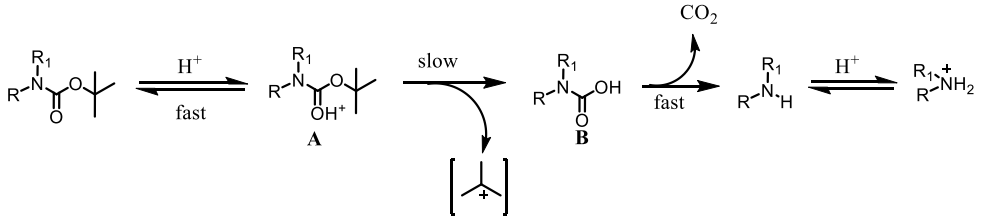
Scheme 2: One step synthesis of impurity B



Scheme 3: Synthesis of impurity B

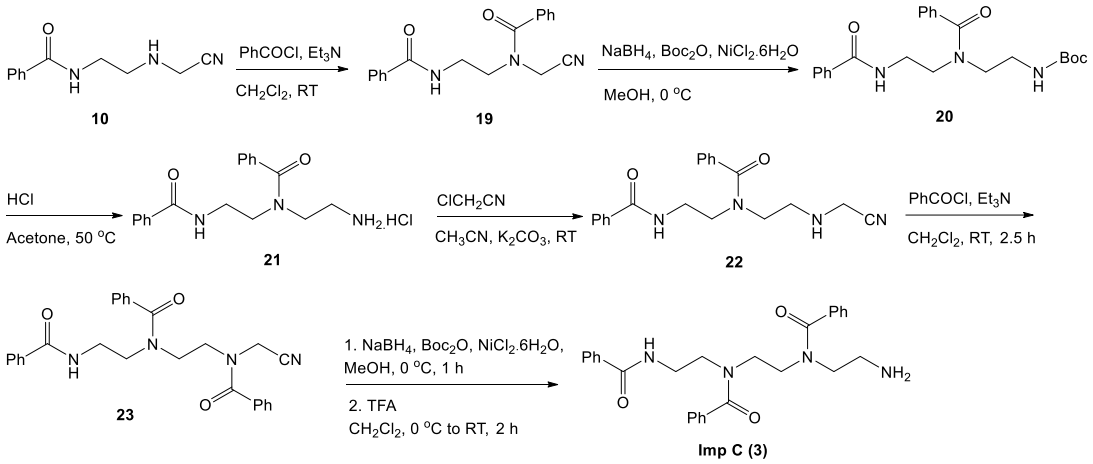
Having encountered a setback, we ensured to follow a safe route to synthesize impurity **B**. We converted ethylenediamine to compound **16** [30] through a one pot two step procedure where we alkylated ethylenediamine with 2-chloroacetonitrile followed by *tert*-butoxycarbonyl protection [22] (Scheme 3). Reduction [22] of dicyano intermediate by Raney Ni to diamine**17** [30] was not smooth enough in our hands. However, we proceeded with the moderate yield. Di-*N*-benzoylation using benzoic anhydride in the presence of triethylamine as acid scavenger afforded compound **18**. Finally, removal of *tert*-butoxycarbonyl group using TFA [27] afforded impurity **B** in substantial yield (Scheme 3).

The widely accepted mechanism for the acid-catalyzed deprotection of a Boc-protected amine is a rapid pre-equilibrium protonation of the Boc group, followed by a ratelimiting fragmentation of the resultant protonated intermediate **A** (Scheme 4)[31,32]. In this mechanism it is assumed that the breakdown of the carbamic acid **B** initially produced by the reaction is fast [33]. For the reaction to be acid catalyzed the liberated tert-butyl cation must undergo further reaction to liberate a proton. In reality the deprotection reaction will consume an equivalent of acid through the protonation of the product amine.



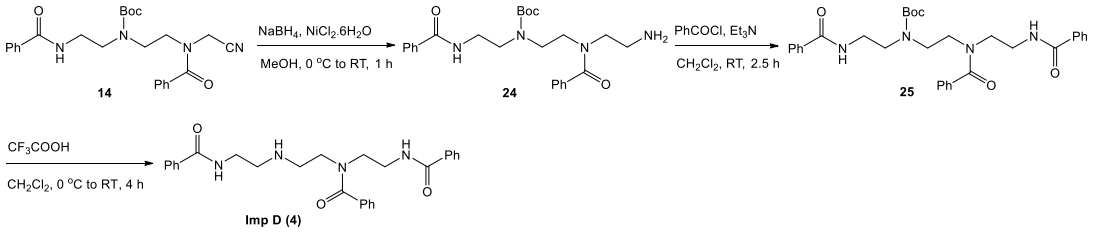
Scheme 4: Expected General Mechanism for the TFA-Catalyzed Deprotection of a Boc Protected Amine

Our next effort was to synthesize impurity **C**. The intermediate compound **10** (from Scheme 1) was benzoylated using reported procedure [25,26] to obtain compound **19** which on reduction [24] with $NaBH_4$ in presence of di-*tert*-butyl dicarbonate afforded compound **20**. Removal [28] of Boc group from compound **20** using hydrochloric acid provided compound **21** which upon alkylation [22] afforded compound **22**. The compound **22** was benzoylated using benzoyl chloride [25,26] to afford compound **23** which on reduction [24] with $NaBH_4$ in presence of di-*tert*-butyl dicarbonate followed by removal of Boc group using TFA [27] afforded impurity **C** (**3**) (Scheme 5).

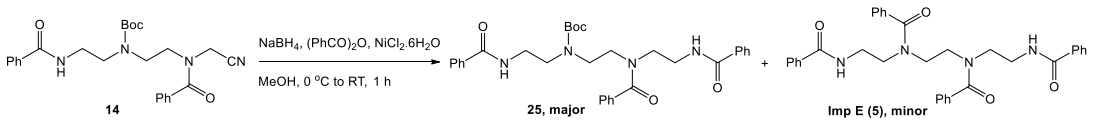


Scheme 5: Synthesis of impurity C

Compound **14** (from Scheme 1) was reduced with $NaBH_4$ using a modified method of Caddick and co-workers [24] to produce intermediate **24**. In this case also we intentionally avoided the addition of di-*tert*-butyl dicarbonate so that we could obtain free amine which was suitable for chain elongation. Intermediate **24** was benzoylated using reported procedure [25,26] to obtain compound **25** (Scheme 6) which finally afforded impurity **D** in substantial yield on treatment with TFA [27].

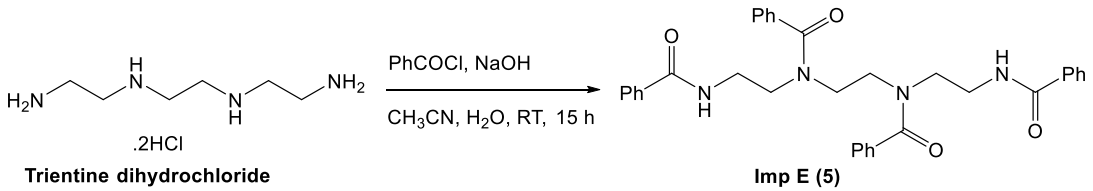


Scheme 6: Synthesis of impurity **D**



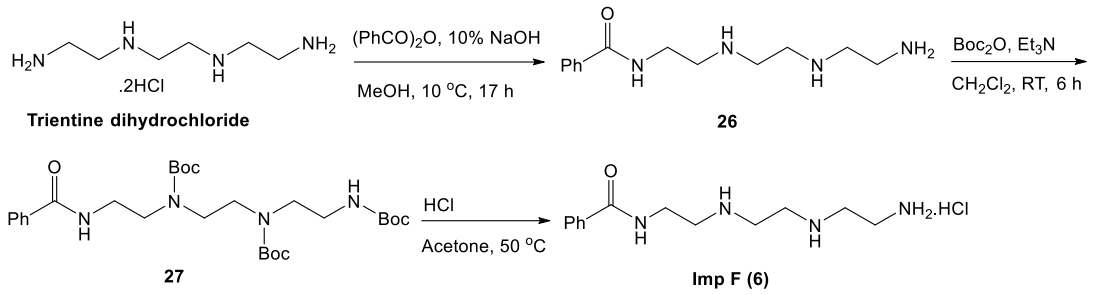
Scheme 7: Accidental synthesis of impurity E

When we tried reduction of compound **14** with $NaBH_4$ in presence of benzoic anhydride to obtain compound **25**, we also obtained perbenzoylated impurity **E** in minor quantity, along with compound **25**, presumably because of Boc removal *in situ* followed by benzoylation of free –NH group (Scheme 7). Same impurity **E** could directly be synthesized by complete benzoylation of Trientinedihydrochloride (Scheme 8).



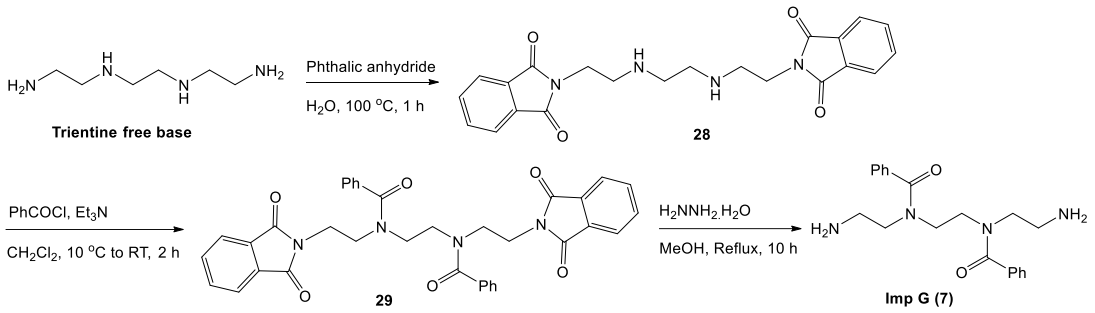
Scheme 8: Synthesis of impurity E

We then switched our focus onto the impurity **F**. Since only one terminal primary amine group was to be benzoylated, we assumed to have a selective benzoylation on terminal nitrogen using controlled condition. Indeed our speculation worked when we reacted Trientinedihydrochloride with 25 mol% of benzoic anhydride using 10% sodium hydroxide to obtain compound **26**. However compound **26** onpurification could not be made free from different other by-products as those impurities also were eluted out of the column together. Therefore, we converted impure **26** to **27** by *tert*-butoxycarbonyl protection [23] which was easily purified using silica gel column. On treatment of pure **27** with conc. hydrochloric acid in acetone at room temperature afforded the impurity **F** in 96% yield with HPLC purity of 91% (Scheme 9).

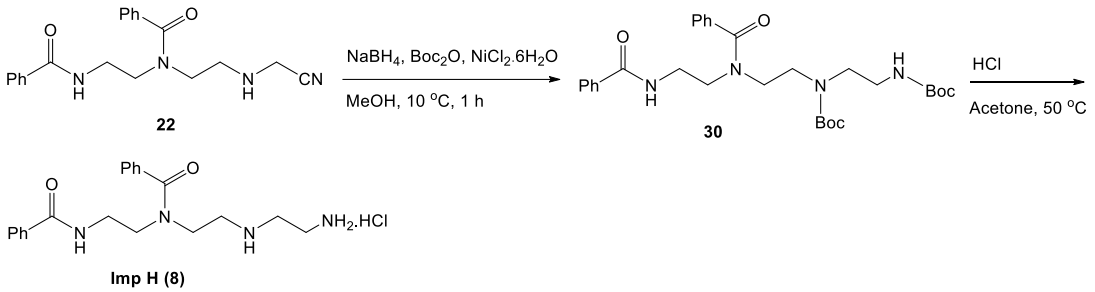


Scheme 9: Synthesis of impurity F

Treatment of TETA with phthalic anhydride [34] in water at 100 °C selectively protected at two terminal ends in the form of bisphthalimide to produce a known [35] compound **28**. Subsequently the compound **28** was per-benzoylated using benzoyl chloride [25,26] in dichloromethane in the presence of triethylamine base to give intermediate **29** which, following Roth's technique of phthalimidedeprotection using hydrazine hydrate [36] in boiling methanol afforded the desired impurity **G** (**7**) (Scheme 10).



Scheme 10: Synthesis of impurity G



Scheme 11: Synthesis of impurity H

Reduction [24] of the intermediate compound **22** (from Scheme 5) with $NaBH_4$ in presence of di-*tert*-butyl dicarbonate afforded compound **30** which on deprotection [28] using hydrochloric acid afforded impurity **H** (**8**) (Scheme 11) as hydrochloride salt.

CONCLUSION

In summary, we have developed first synthetic routes for impurities**A, B, C, D, E, G** and **H** related to Trientinealong with new synthetic route for the synthesis of impurity **F**.Following systematic and practically viable process and using cheap raw materials we could achieve moderate to good overall yields of all the impurities.

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