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Novel high yielding route for the synthesis of Melphalan dimer impurity G

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Melphalan dimer is an impurity from the synthesis of Melphalan (M216900) which is an antineoplastic agent. In this article, a new route for the synthesis of Melphalan dimer impurity G has been discussed. The new research provides the first high-yield, high-purity synthesis of this impurity. The impurity has been confirmed for purity using HPLC, and its structure has been identified using MS, ^{1}H and ^{13}C NMR.

Keywords: Melphalan, Impurity, Synthesis, Dimer impurity, Antineoplastic agent

Melphalan was first used in the late 1950s in therapeutic use[1]. Bergel and Stock were the first to synthesise Melphalan in 1953. Melphalan chemically is 4-[bis (2-chloroethyl) amino]-L-phenylalanine[2], a derivative of the nitrogen mustard (L-PAM) and L-isomer of Melphalan is active. It is a chemotherapeutic drug that is commonly used to treat cancer. It works against multiple myeloma, lymphomas, malignant melanoma, and ovarian cancer by creating covalent connections with nitrogenous bases of the DNA, accumulation of an alkyl group to DNA component, and generating a DNA cross-linking[3].

Pullagurla *et al*. reported the synthesis of Melphalan and its hydrochloride salt[4]. Organic, inorganic, and residual solvent impurities are the three categories of impurities defined by the ICH rules. The United States Pharmacopoeia and the British Pharmacopoeia have both highlighted probable contaminants in Melphalan hydrochloride API. For quality and safety reasons, identifying such impurities is critical. The reported impurities in the British Pharmacopoeia are Melphalan impurity H (**1**), Melphalan impurity I (**2**), Melphalan impurity isopropyl ester (**3**), Melphalan impurity J (**4**), Melphalan impurity D-isomer (**5**), Melphalan impurity A (**6**), Melphalan impurity B (**7**), Melphalan dimer impurity G (**8**) as shown in Fig. 1

All of these impurities have an important influence on the quality of the pharmaceutical product and hence this work is extremely valuable to the generic pharmaceutical industry. Because impurity separation is a time-consuming process that yields insufficient amounts, impurity synthesis allows for the use of a pure form of impurities in the identification of their biological effects and may be used as a standard marker to detect the occurrence of identical impurities in active pharmaceutical ingredients.

Among these Melphalan Dimer **8** is a significant impurity sought by regularity agencies. The procedure for the synthesis of Melphalan dimer hydrochloride salt has been published[5]. However, poor yield and difficulty in isolating the final product necessitate the development of a new approach with a better yield and purity of the end product[6]. The unique high-yielding synthesis approach of the Melphalan dimer impurity G trifluoroacetic acid salt form is reported here.

Results and Discussion

The synthesis of the dimer impurity G was shown in Scheme 1. Compound **10** was synthesized by esterifying compound **9** with methanol and hydrochloric acid. Compound **11** was formed by protecting the amine **10** by protecting group tert-butoxy carbonyl in tetrahydrofuran (THF) and sodium bicarbonate ($NaHCO_3$). The compound **11** was then carboxylated in the presence of sodium acetate (NaOAc), dimethylformamide (DMF), and sodium carbonate (Na_2CO_3) to form compound **12**. Compound **13** was formed by deacetylating compound **12** with NaOH and methanol as a solvent. In compound **13**, alcohol was

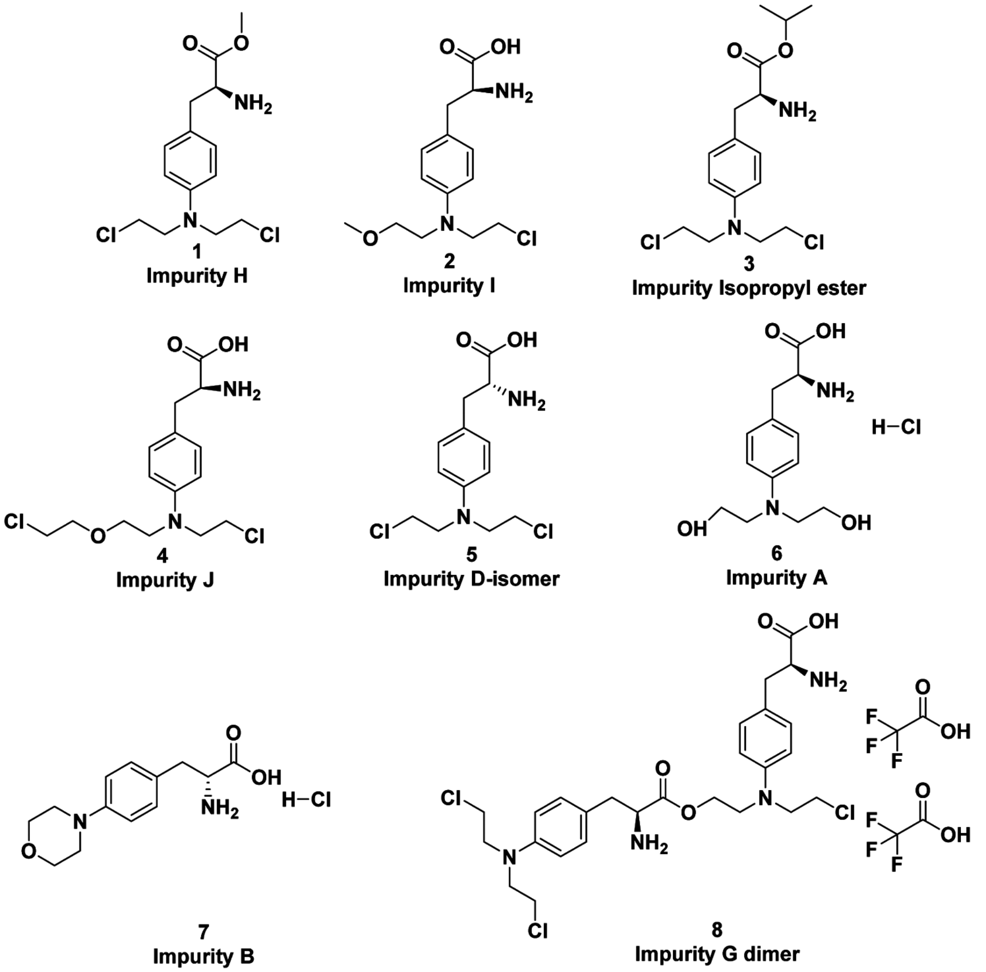


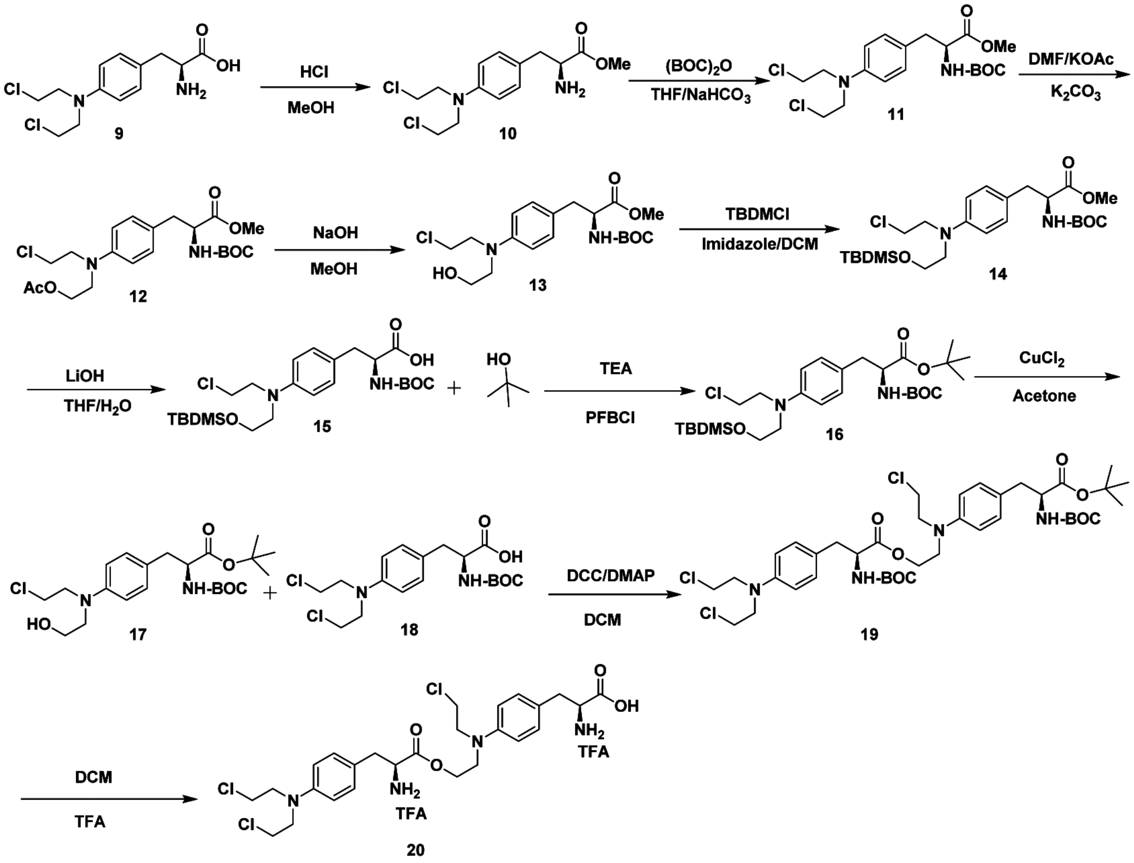
Fig. 1 — Impurities of Melphalan

protected by utilizing the protective group t-butyldimethylsilylchloride (TBDMCl) in presence of imidazole, dichloromethane (DCM) to generate compound **14**. The ester of compound **14** was demethylated in the presence of KOH, tetrahydrofuran (THF), and water to yield compound **15** as an acid product. Compound **16** was synthesized by incorporating an isobutyl group at an acidic proton in compound **15** using tert-butanol, triethylamine (TEA), and pentafluorobenzoyle chloride (PFBCl). Compound **17** was prepared by deprotecting the tert-butyldimethylsilyl group of compound **16** with copper chloride in acetone-water. Compound **19** was produced by condensing compounds **17** and **18** using dichloromethane (DCM), 1-Ethyl-3-[3-dimethyl-aminopropyl] carbodiimide (EDC), and 4-dimethyl-aminopyridine (DMAP). Finally, product **20** was synthesized by stirring compounds with HCl in dioxane.

Experimental Details

Synthesis of methyl-(S)-2-amino-3-(4-(di(2-chloroethyl)amino) phenyl)propanoate, 10

Dried methanol (75 mL) was combined with dried and purified **9** (4.5 g, 0.014 mol). Hydrochloric acid gas (15 g) was purged into the dry reaction mixture. Then reaction mass was heated around 65°C, and the process was accelerated by removing the water created in the reaction by using Dean-Stark apparatus. After completing the esterification processas indicated by TLC, the reaction mass was neutralised using $NaHCO_3$, and the precipitated inorganic salt was separated through filtration. The filtrate was collected and distilled to get a thick mass of the product **10** (Product yield 4.32 g, 96%)[7]. m.p.213-215°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.83 (2H, d), 3.50 (4H, t), 3.59 (1H, t), 3.71 (3H, s), 3.92 (4H, t), 6.66 (2H, d), 6.84 (2H, d), 8.73 (2H, s).



Scheme 1 — Synthesis of Melphalan dimer impurity G

MS: *m/z* Calcd for compound **10** $C_{14}H_{20}Cl_2N_2O_2$ [M+H]: 319.09. Found 319.10.

Synthesis of methyl-(S)-3-(4-(di(2-chloroethyl)amino) phenyl)-2-((tert-butoxycarbonyl)amino)propanoate, 11

Di-tert-butyl dicarbonate $(BOC)_2O$ (4.22 g, 0.018 mol) was added dropwise at 0°C to a stirred solution of compound **10** (4.1 g, 0.012 mol) in THF/ sat. $KHCO_3$ solution (1:1) (60 mL). After complete addition, the reaction mass was diluted with 80 mL of water and extracted with ethyl acetate twice (60 mL). The total organic layer was mixed, dehydrated over anhydrous Na_2SO_4, filtered, and evaporated to get a crude product **11**. The above product was purified using column chromatography (10% EtOAc/diethyl ether) to obtain pure compound **11** (Product yield 3.64 g, 88.88%) as an off bleached solid[8]. m.p.284-286°C. ^1H NMR (400 MHz, DMSO-d_6): δ 1.43 (9H, s), 2.77, (2H, d), 3.50 (4H, t), 3.71 (3H, s), 3.91 (4H, t), 4.70 (1H, t), 6.66 (2H, d), 6.84 (2H, d), 7.35 (1H, s); MS: *m/z* Calcd for compound **11** $C_{19}H_{28}Cl_2N_2O_4$ [M+H]: 419.14. Found: 419.11.

Synthesis of methyl-(S)-3-(4-((2-acetoxyethyl)(2-chloroethyl) amino)phenyl)-2-((tert-butoxycarbonyl)amino)propanoate, 12

Compound **11** (3.5g, 0.009 mol) and Na_2CO_3 (3.0 g, 0.028 mol) was mixed in DMF (50 mL) until completely dissolved. To the reaction flask NaOAc (1 g, 0.003 moles) was added at ambient temperature and the reaction flask was further heated up to 90°C, for 6 h. TLC was checked up to the completion of the reaction. The reaction mass was diluted with 50 mL of water and extracted with ether twice (40 mL), Total organic layer was collected and dehydrated over Na_2SO_4. The above raw product was purified using column chromatography, with 15% EtOAc/Pet-ether to get pure compound **12** (Product yield 3.19 g, 91.23%). m.p.319-321°C. ^1H NMR (400 MHz, DMSO-d_6): δ 1.43 (9H, s), 2.05 (3H, s), 2.77 (2H, d), 3.40(2H, t), 3.50 (2H, t), 3.71 (3H, s), 3.91 (2H, t), 4.47 (2H, t), 4.71 (1H, t), 6.66 (2H, d), 6.84 (2H, d), 7.36 (1H, s); MS: *m/z* Calcd for compound **12** $C_{21}H_{31}ClN_2O_6$ [M+H]: 443.19. Found: 443.21.

Synthesis of methyl-(S)-2-((tert-butoxycarbonyl) amino)-3-(4-((2-chloroethyl)(2-hydroxyethyl)amino)phenyl)propanoate, 13

Compound **12** (3.0 g, 0.0079 mol) was added to methanol (150 mL) and aq. NaOH (2 mL, 25%). The reaction mass was warmed up to 80°C and then refluxed for 22 h. The solution was chilled and neutralized with hydrochloric acid. After completion of the reaction as indicated by TLC, the solvent was removed under low pressure to yield the raw product. The raw compound was diluted with 45 mL of water and extracted with ethyl acetate (50 mL). Total organic layers were collected and dehydrated over Na_2SO_4, filtered, and concentrated. The above crude product was purified using column chromatography, with 15% EtOAc/Pet-ether to obtain the pure compound **13** (Product yield1. 2.44 g, 81.4%)[9]. m.p.320-322°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.43 (9H, s), 2.77 (2H, d), 3.33 (2H, t), 3.46 (2H, t), 3.60 (2H, t), 3.71 (3H, s), 3.91 (2H, t), 4.71 (1H, t), 6.66 (2H, d), 6.84 (2H, d), 7.39 (1H, s); MS: *m/z* Calcd for compound **13** $C_{19}H_{29}ClN_2O_5$ [M+H]: 401.18. Found: 401.24.

Synthesis of methyl (S)-2-((tert-butoxycarbonyl) amino)-3-(4-((2-((tert-butyldimethylsilyl)oxy)ethyl)(2-chloroethyl)amino)-phenyl)propanoate, 14

The compound **13** (2.2 g, 6.5 mmol), N-methylimidazole (0.25 g, 3 mmol), and iodine (0.5 g, 2 mmol) were dissolved in solvent THF (4 mL). Then TBDMSCl (0.17 g, 1.12 mmol) was added, and the reaction mass was stirred for 2-3 h at ambient temperature till the reactant was completely gone on TLC. The solvent was removed, the residue was mixed in EtOAc, and the mixture was washed with aq. sodium thiosulphate. The organic phase was dehydrated over anhydrous Na_2SO_4 and evaporated. The compound was purified using silica-gel column-chromatography to get the pure product **14** (Product yield 4.9 g, 96%)[10]. m.p.330-332°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.2 (6H, s), 0.98 (9H, s), 1.43 (9H, s), 2.77 (2H, d), 3.36 (2H, t), 3.38 (2H, t), 3.50 (2H,t), 3.71 (3H, s), 3.92 (2H,t), 4.70 (1H, t,), 6.66 (2H, d), 6.81 (2H, d), 7.35 (1H, s); MS: *m/z* Calcd for compound **14** $C_{25}H_{43}ClN_2O_5Si$ [M+H]: 515.26. Found: 515.27.

Synthesis of (S)-2-((tert-butoxycarbonyl)amino)-3-(4-((2-((tert-butyldimethylsilyl)oxy)ethyl)(2-chloroethyl)amino)-phenyl)propanoic acid, 15

To a solution of **14** (4.0 g, 8.9 mmol) in methanol (44 mL), 2N KOH (44 mL) was added at 0°C. To dissolve the precipitate formed, 40 mL of THF was added. The solution was stirred for 3 h at 0 °C. After completing the reaction as confirmed by TLC, the mixture was extracted with ethyl acetate three times after acidifying the mixture with saturated citric acid (pH 4). The combined organic phase was dried over Na_2SO_4 and evaporated. The residue was filtered and dissolved in CH_2Cl_2, to obtain the product **15**, after evaporation[11]. (Product yield 3.70 g, 92.57%). m.p.335-337°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.22 (6H s), 0.78 (9H, s), 1.43 (9H, s), 2.77 (2H, d), 3.36 (2H, t), 3.38 (2H, t), 3.50 (2H, t), 3.92 (2H, t), 4.75 (1H, t), 6.66 (2H, d), 6.84 (2H, d), 7.39 (1H, s), 12.80 (1H, s); MS: *m/z* Calcd for compound **15** $C_{24}H_{41}ClN_2O_5Si$ [M+H]: 501.25. Found: 501.32.

Synthesis of isopropyl (S)-2-((tert-butoxycarbonyl) amino)-3-(4-((2-((tert-butyldimethylsilyl)oxy)ethyl)(2-chloroethyl)amino)-phenyl)propanoate, 16

PFBCl (0.28 g, 1.20 mmol), tert-butanol (0.22 g, 3 mmol), and triethylamine (0.4 g, 4 mmol) were added to a solution of **15** (2.5 g, 5.76 mmol) in dry toluene (10 mL) and the reaction mass was stirred at 110°C for 36 h. The solvent was removed by evaporation. The crude compound was dissolved in ethyl acetate (20 mL) and washed with a saturated solution of $NaHCO_3$ and brine, dried over anhydrous $MgSO_4$, and filtered. The organic solvent was removed by evaporation and purified by flash column chromatography (95/5 PE/EtOAc) to get a pale creamy solid **16**[12]. (Product yield 1.75 g, 70.11%). m.p.330-332°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.22 (6H,), 0.78 (9H, s), 1.39 (9H, s), 1.45 (3H, d), 2.77 (2H, d), 3.36 (2H, t), 3.38 (2H, t), 3.50 (2H, t), 3.92 (2H, t),4.71 (1H, t), 4.82 (1H, m), 6.66 (2H, d), 6.81 (2H, d), 7.39 (1H, s); MS: *m/z* Calcd for compound **16** $C_{27}H_{47}ClN_2O_5Si$ [M+H]: 543.29. Found: 543.32.

Synthesis of isopropyl (S)-2-((tert-butoxycarbonyl) amino)-3-(4-((2-chloroethyl)(2-hydroxyethyl)amino) phenyl)propanoate, 17

The compound **16** (2.1 g, 4.2 mmol) was mixed in acetone/H_2O (95/5, 10 mL) and $CuCl_2.H_2O$ (6.72g, 0.05 mmol) was added to the mixture, and the solution was warmed under mild reflux for 5-6 h until reaction was completed, as indicated by a TLC. The solvent was evaporated, and the residue was treated to silica-gel column-chromatography (Product yield 2.05 g, 98%, **17**)[13]. m.p.322-324°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.39 (9H, s), 1.45 (6H, d), 2.77 (2H, d), 3.33 (2H, t), 3.46 (2H, t), 3.60 (2H, t), 3.91 (2H, t), 4.71 (1H, t), 4.82 (1H, m), 6.66 (2H, d), 6.84 (2H, d), 7.39 (1H, s); MS: *m/z* Calcd for compound **17** $C_{21}H_{33}ClN_2O_5$ [M+H]: 429.21. Found: 429.26.

Synthesis of 2-((4-((S)-2-((tert-butoxycarbonyl)amino)-3-isopropoxy-3-oxopropyl)phenyl)(2-chloroethyl) amino)ethyl (S)-3-(4-(bis(2-chloroethyl) amino) phenyl)-2-((tert-butoxycarbonyl) amino) propanoate, 19

Compound **17** (0.328 g, 0.76 mmol) and compound **18** (0.30 g, 0.74 mol) were dissolved in DCM (7 mL) and mixed well, further addition of EDC (0.168 g, 0.87 mmol) was carried out at 0°C. Then the reaction mixture was stirred for 3 h at ambient temperature followed by the addition of DMAP (4.4 mg, 0.01 mmol). TLC was checked for completion of the reaction and after completing the reaction, the solvent was distilled to obtain crude compound **19**. The above compound was mixed in ether (45 mL) and filtered with a sintered funnel. The organic layer was rinsed with $KHCO_3$ solution (20 mL), brine solution (15 mL), and dehydrated over MgSO4 and solvent evaporated to get concentrated crude compound **19**. The above crude compound was refined by column-chromatography, with only 15% ethyl acetate/Pet-ether mixed total fractions eluted and concentrated to get pure compound **19** as a light-yellow sticky solid (Product yield 0.44 g, 70.71%). m.p.360-362°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.39 (9H, s), 1.40 (9H, s), 1.45 (6H, d), 2.75 (2H, d), 2.77 (2H, d), 3.40 (2H, t), 3.50 (4H, t), 3.51 (2H, t), 3.92 (6H, t), 4.47 (2H, t), 4.70 (1H, t), 4.71 (1H, t), 4.82 (1H, m), 6.66 (2H, d), 6.66 (2H, d), 6.81 (2H, d), 6.82 (2H, d), 7.34 (1H, d), 7.39 (1H, s); MS: *m/z* Calcd for compound **19** $C_{39}H_{57}Cl_3N_4O_8$[M+H]: 815.32. Found: 815.36.

Synthesis of (S)-2-amino-3-(4-((2-(((S)-2-amino-3-(4-(bis(2-chloroethyl)amino)phenyl)propanoyl) oxy)ethyl)(2-chloroethyl)amino)phenyl)propanoic acid. 2,2,2-trifluoroacetic acid, 20

Compound **19** (0.32 g, 0.39 mmol) was dissolved in dioxane (40 mL) and 4M HCl (10mL) was added to it at room temperature. The reaction mixture was then stirred for 24 hours. After completion of the reaction as indicated by TLC, the solvent was evaporated to yield compound **20**. The above compound was cleaned with ether (12 mL), DCM (12 mL), and purified by preparative TLC (5 % MeOH/DCM) to get pure compound **20** (Product yield 0.23 g, 75%). m.p.346-348°C. ^{1}H NMR (400 MHz, DMSO-d_6): δ 2.89-2.95 (4H, d), 3.64-3.68 (16H, t), 4.05-4.07 (2H, t),4.19-4.20 (2H, t), 6.64-6.71 (4H, d), 7.00-7.08 (4H, d), 8.23-8.25 (4H, s); ESI-MS: *m/z* Calcd for compound **20** $C_{30}H_{37}Cl_3F_6N_4O_8$ [M+H]: 573.17. Found: 575. HPLC purity 99.59%.

Conclusion

In conclusion, this study provides the first high-yield, high-purity synthesis of Melphalan dimer impurity. The impurity was confirmed for purity using HPLC, and its structure was identified using MS, ^{1}H, and ^{13}C NMR techinques.

Supplementary Information

Supplementary information is available in the website http://nopr.niscpr.res.in/handle/123456789/58776.

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