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Bulk Synthesis of Thermally Unstable 5-Amino-3-methylisoxazole Using Silica Coated Magnetite Nanoparticles ($Fe_3O_4@SiO_2$)

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5-Amino-3-methylisoxazole is an important intermediate in medicinal chemistry. Bulk scale synthesis of it is reported and the reaction yields are typically around 40 %. The reaction of 3-aminocrotonitrile and hydroxylamine hydrochloride in aqueous environment in about 12 h at room temperature results in 5-amino-3-methylisoxazole. This simple one pot synthesis has an issue, which contributes to lower reaction yields. The melting point of 5-amino-3-methylisoxazole is 83-85 °C, but around 70 °C it can rapidly decompose causing an explosion. Hence, this molecule is synthesized by maintaining reaction temperature at 0-25 °C. Due to exothermic nature of the reaction, maintenance of lower temperature is mandatory. Due to lower reaction temperature, this reaction yields are usually low. Though these kind of lower yields are reported for synthesis of this class of compounds, attempts to improve the reaction yields are sparse. We report silica coated magnetic nanoparticle ($Fe_3O_4@SiO_2$) as an inert surface for preparation of 5-amino-3-methylisoxazole. The yield of reaction is around 80 %, almost double the reported yield and it is enabled by the 40 nm $Fe_3O_4@SiO_2$ particles. The 40 nm $Fe_3O_4@SiO_2$ are synthesized by modification to the famous "Stober" protocol. The obtained 40 nm particles contain intermittent particles with about 200 nm as reported in literature. Though isolation of 40 nm size magnetic nanoparticles in reactions is difficult, presence of about 200 nm size $Fe_3O_4@SiO_2$ is enabling for easy isolation. The work up of reaction is simple isolation of $Fe_3O_4@SiO_2$ by magnet, basification of reaction mixture to pH 10, dissolving the precipitated solid in diethyl ether, removal of ether to obtain crude 5-amino-3-methylisoxazole, which was recrystallized using distilled water. This protocol paves way for simple synthesis of related isoxazoles, which are increasingly forming base structure for many drugs and medicinal chemistry leads.

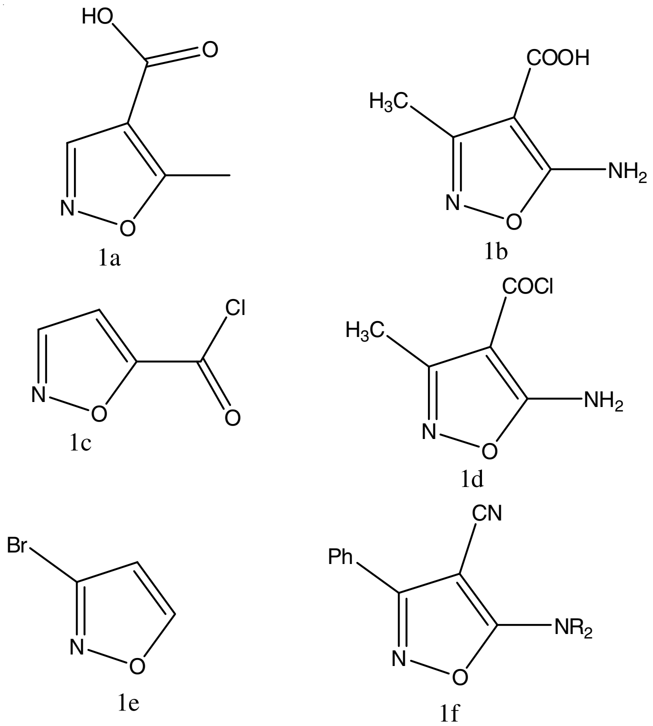
Keywords: 5-Amino-3-methylisoxazole, Magnetite nanoparticles, $Fe_3O_4@SiO_2$, Silanization, Tetraethyl orthosilicate.

INTRODUCTION

Isoxazoles are an interesting functional group for medicinal chemistry [1-4], bringing two hetero atoms to the drug molecule. Though excess presence of heteroatoms in molecule violates Lipinski rule of five [5-8], now most medicinal chemistry leads are based on heteroatoms [9-12]. 5-Amino-3-methylisoxazole [13-15] is a starting material mostly used for introduction of isoxazole ring on a lead molecule. Fig. 1 shows the isoxazole base ring with functional groups: acid (1a, 1b), acid chloride (1c, 1d), bromo (1e), cyano (1f), *etc*.

Scheme-I shows the most favoured synthetic route for 5-amino-3-methylisoxazole. The starting materials are hydroxylamine hydrochloride [16-18] and 3-aminocrotonitrile [19-21] and are used in a one pot synthesis in aqueous environment. Reaction is typically performed for overnight, maintaining 0-25 °C and the typical reaction yields are 40-50 %. Though catalysts for similar cyclization reactions are reported [22,23], this molecule did not have much reported methods for improvement of its yield. The mechanism as shown in **Scheme-II**, requires the N-O bond formation and is a rate limiting step.

Use of solid supports for isoxazole ring cyclization are less known and silica coated magnetic nanomaterials ($Fe_3O_4@SiO_2$) are not reported in literature. $Fe_3O_4@SiO_2$ is an inert material and around 100 nm size are widely used in organic chemistry as solid support [24-27]. $Fe_3O_4@SiO_2$ is especially useful for lowering reaction temperature [28], improving yields [29,30], elimination of reaction steps [31] and reported to catalyze formation of some key organic products [32,33]. In case of 5-amino-3-methylisoxazole, the molecule melts at 82-84 °C, but occasionally decomposes explosively at around 70 °C [34]. Hence, the reaction cannot be allowed to cross room temperature due to exothermic nature of condensation of hydroxylamine and 3-aminocrotonitrile. The maintenance of 0-25 °C reaction temperature reduces the yield of the reaction and is thermodynamically maintained at around 50 %. The aim of this study

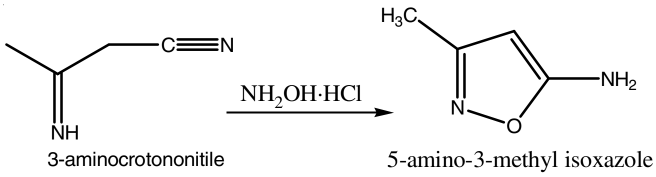


1a 1b

1c 1d

1e 1f

Fig. 1. Common medicinal chemistry precursors for introduction of isoxazole ring in leads



Scheme-I: Reaction of 5-amino-3-methylisoxazole

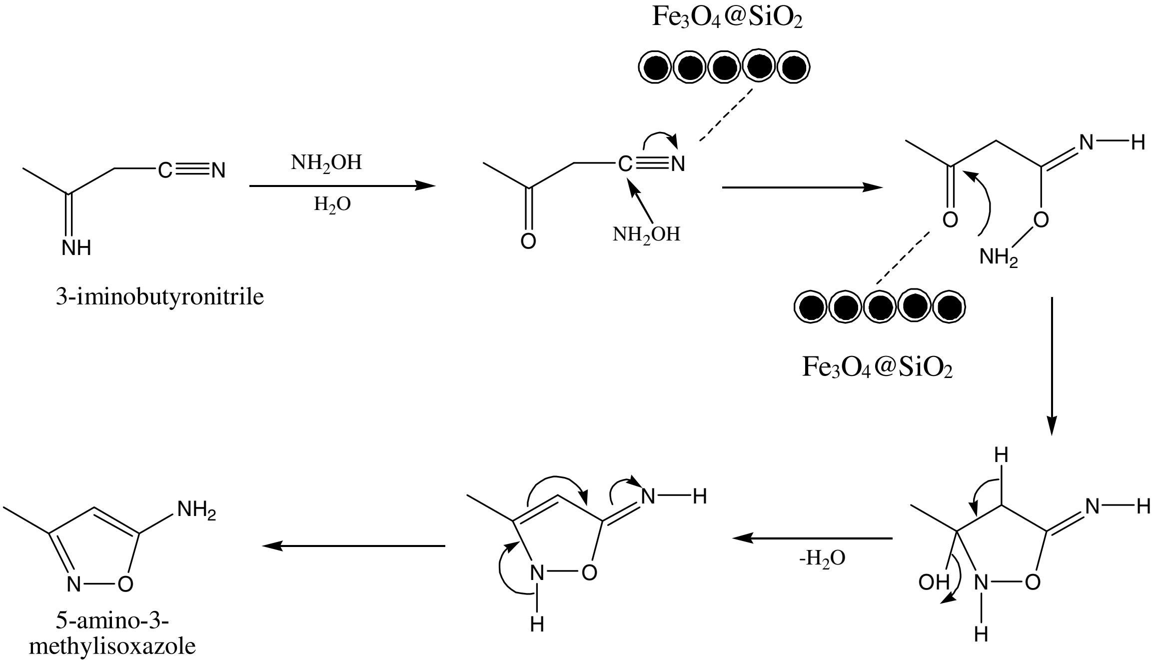
is to improve reaction yields to around 80 % using reusable 40 nm size Fe$_3$O$_4$@SiO$_2$ particles in a one pot synthesis.

EXPERIMENTAL

All the starting materials are laboratory reagent grade with minimum of 98 % purity and purchased from Sigma-Aldrich. Organic solvents are of analytical grade and THF used is a dry grade solvent. The magnetite nanoparticle characterization done with IR spectroscopy, SEM and TEM. Organic molecules synthesized by using magnetite nanoparticles were characterized by NMR, Mass spectrometry and elemental analysis.

Synthesis of silanized magnetite nanoparticles

Preparation of magnetite nanoparticles (MNPs): 17 g of sodium nitrate and 10 g of sodium hydroxide were taken in a clean, dry, three necked round bottom flask and to that 950 mL of distilled water was added. This was degassed using argon gas and heated for 30 min on water bath at 90 °C. This mixture was cooled to ≤ 60 °C. In a separate beaker, 1 mL of 2 M sulfuric acid was added to 13.9 g of ferrous sulphate, dissolved, made up to 100 mL. The iron sulfate solution was added to the above mixture slowly with a constant flow rate. Black magnetic nanoparticles formation was observed. Continued the degassing for 5 min and heated for 4 h at 90 °C under argon atmosphere. The hot reaction contents were transferred into a beaker, left on a magnet for 15 min. After the magnetic nanoparticles settled at the bottom of flask and the resultant clear supernatant was decanted. To remove the unreacted sodium hydroxide, distilled water was added, swirled, allowed the contents to settle down and supernatant was discarded. Washes are given till the pH of the medium reached below 7.5



Scheme-II: Mechanism of formation of 5-amino-3-methylisoxazole and role of silica surface in enhancement of rate of ring cyclization. 3-aminocrotonitrile undergoes hydrolysis where imine group converted to ketone group. Ammonium hydroxide attack on carbon of CN group. Lone pair of nitrogen attack on carbonyl carbon. Elimination of water molecule and rearrangement of hydrogen takes place. The silica surface due to its acidic nature polarizes the hetero atom and hence the nucleophilic attack on the parent carbon atom and it indicated complete removal of unreacted sodium hydroxide. The yield obtained is about 10 g.

Silanization of magnetite nanoparticles: In a beaker, 800 mL of ethanol and 200 mL of water were taken, pH is maintained at 12 using sodium hydroxide and was added to magnetite nanoparticles. The solution was sonicated for 60 min for removal of lumps. To the above solution, 10 mL of tetraethyl orthosilicate (dissolved in 20 mL of ethanol) was added. Further sonication was continued for 1 h. The sonicated $Fe_3O_4@SiO_2$ solution was allowed to settle on magnet for 30 min and supernatant was discarded. The particles are washed with distilled water (3 × 200 mL) till the pH was reached below 7.5. Fig. 2 shows the procedure of silanization magnetite nanoparticles.

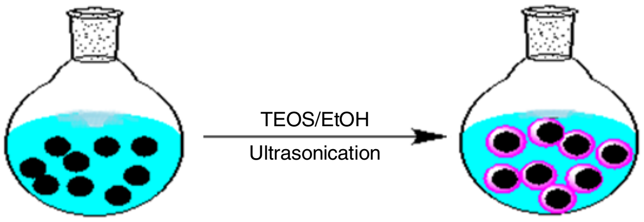


Fig. 2. Conventional silanization procedure for magnetite nanoparticles. The procedure involved addition of TEOS during sonication and the resultant magnetite nanoparticle are of uniform size. The significant deviation from reported silanization procedures is that, the magnetite nanoparticle did not require calcination and the SiO_2 obtained is uniform for all synthetic purposes. To use $Fe_3O_4@SiO_2$ as catalyst for bulk synthesis, elimination of calcination step was very crucial

Removal of traces of Fe^{2+} and Fe^{3+}: The magnetite nanoparticle synthesized by this method has traces of Fe^{3+} or Fe^{2+} ions. The traces of unreacted Fe^{2+} and Fe^{3+} were removed by refluxing in 0.3 M citrate at pH 6 and 10 mM *tris* containing 1 mM EDTA at pH 8. For citrate wash, $Fe_3O_4@SiO_2$ were mixed with 200 mL of 0.3 M citrate buffer (pH 6) in round bottom flask and heated for 0.5 h at 90 °C. After the solution was cooled, water wash is given three times and $Fe_3O_4@SiO_2$ were separated using magnet. For *Tris*-EDTA wash, $Fe_3O_4@SiO_2$ were mixed with 200 mL of 10 mM tris, 1 mM EDTA (pH 8) and heated for 0.5 h at 90 °C. The $Fe_3O_4@SiO_2$ were separated using magnet and washed three times with distilled water. Magnetite nanoparticles were stored in distilled water and prior to use, separated using magnet, dried in oven at 250 °C for 24 h. The obtained $Fe_3O_4@SiO_2$ at this stage are around 40 nm in size and the SEM images were shown in Fig. 3 and TEM images were shown in Fig. 4. Fig. 5 shows IR spectra before and after silanization.

Synthesis of 5-amino-3-methylisoxazole: 186.9 g (2.69 mol) of hydroxylamine hydrochloride and 150 g (1.8 mol) 3-amino crotonitrile was mixed in 3 L of water and taken in flask, stirred for overnight. After 12 h of reaction, the reaction was basified to pH 10 with 25 g of sodium carbonate. The insoluble material was filtered, stirred in 600 mL of ether and filtered. The obtained ether filtrate was combined with aqueous filtrate. The ether layer was separated and the aqueous layer was extracted with ether (2 × 300 mL). The ether layer was combined, dried over anhydrous $MgSO_4$ (125 g), filtered and stripped under vacuum to leave a solid which was dried over KOH under vacuum. The solid was recrystallized from water to give cream (pale pink) fine crystalline solid which was collected on porcelain and washed with chilled water and dried. Melting point reported 83-85 °C, obtained 82-84 °C. Yield 50 %.

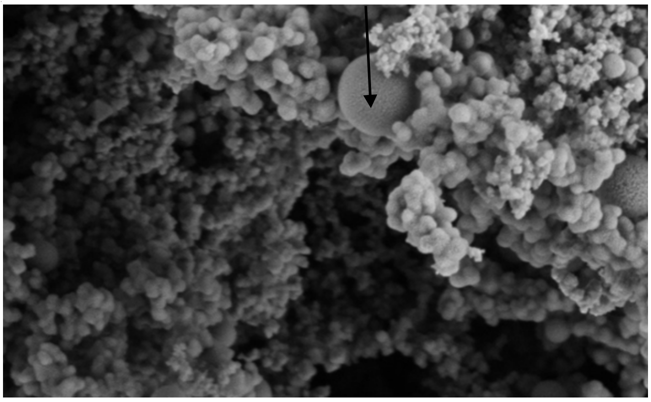


Fig. 3. SEM image of $Fe_3O_4@SiO_2$ magnetic nanoparticles. The magnetite nanoparticle are not coated with any surfactant and the aggregation observed is expected. A mere mechanical stirring or ultra-sonication in a solvent is used to suspend these particles just before the reaction. The arrow marked magnetite nanoparticle are about 180 nm particles and rest of the particles are of 40 nm size. The role of 180 nm particle is increase the magnetism to isolate particles during the reaction

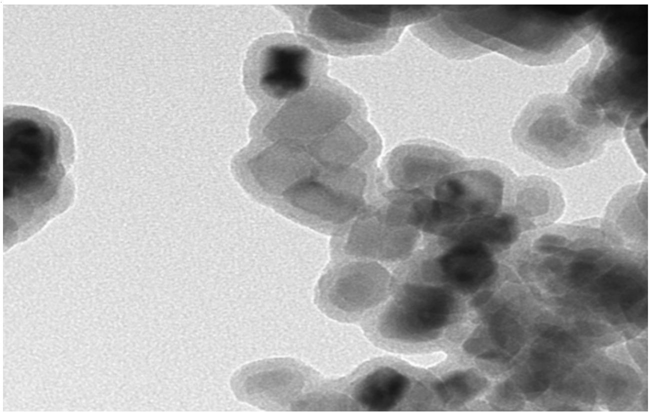


Fig. 4. TEM image of synthesized 40 nm magnetic nanoparticles. The dark structure is the magnetic core and the light coloured layer is the silica coating. The ideal performance of 40 nm particle for bulk scale synthesis was probably obtained because of the appropriate magnetism and improved surface area

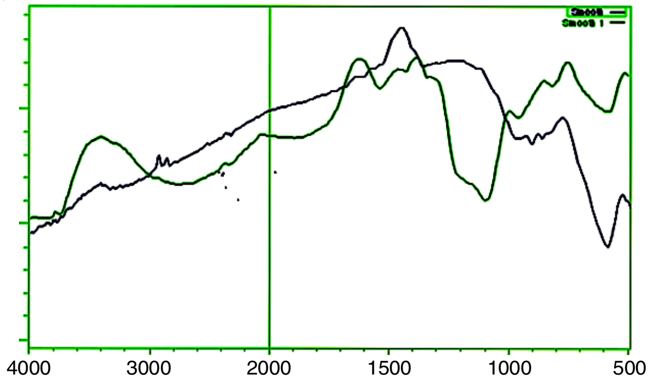


Fig. 5. IR spectra of magnetite nanoparticles before and after silanization. Infrared (IR) spectral characterization of silanized (Blue) and non-silanized magnetite nanoparticle (Green). The 1100-1200 cm^{-1} peak is characteristic of silanization and as reported it is one of the easiest ways to characterize

Synthesis of 5-amino-3-methylisoxazole using 40 nm size Fe_3O_4@SiO_2: 186.9 g (2.69 moles) of hydroxylamine hydrochloride, 150 g (1.8 mol) 3-amino crotonitrile and 30 g (1 % w/v) Fe_3O_4@SiO_2 was mixed in 3 L of water stirred for overnight in flask. After 12 h of reaction, the magnetic nanoparticles were removed using an external barium ferrite magnet. The reaction mixture was alkalized to pH 10 using 25 g of sodium carbonate. The mixture was filtered, the solids are stirred with 600 mL of ether and filtered. The obtained ether filtrate was combined with aqueous filtrate, ethereal layer was separated. The aqueous layer was extracted with ether (2 × 300 mL), the ether layer was dried over anhydrous $MgSO_4$ (125 g), organic solvent stripped under vacuum to leave a solid which was dried over KOH under vacuum. The solid was recrystallized from water to give cream (pale pink) fine crystalline solid which was collected on porcelain, washed with cold water and dried. Melting point reported 83-85 °C, observed 82-84 °C. Yield 80 %, ^{1}H NMR ($CDCl_3$, 400 MHz): 2.25 (s, 3H, CH_3), 4.9 (s, 2H, NH_2), 5.0 (s, 1H, CH). ^{13}C NMR ($CDCl_3$, 400 MHz): 168.4, 164, 96.5 and 14.2, Mass 97.1 (M+1), Elemental anal: $C_4H_6N_2O$ (%): C-48.97, H-6.16, N-28.56 and O-16.31. Found: C-48.4, H-5.98, N-28.2 and O-16.28.

RESULTS AND DISCUSSION

Synthesis and characterization of Fe_3O_4@SiO_2: Magnetite (Fe_3O_4) nanoparticles are synthesized by a modification of Stober's method [35]. Silanization of Fe_3O_4 was performed using TEOS/EtOH system using ultra sonication. The SEM image (Fig. 3) shows that the typical size of nanoparticles is around 40 nm. There are about 180 nm big particles occasionally present as shown in Fig. 3 and it reported that during nucleation, this does happen with magnetic particles [36]. We also use mild mixing during nucleation step for obtaining majority of 40 nm particles. In this process, we provide a fine stream of nitrogen/argon gas and this could have resulted in non-uniform nucleation at the walls of the round bottom flask causing the formation of 180 nm particles.

The Fe_3O_4 core and the uniform silica coating are visible in TEM image shown in Fig. 4. This is one of the few instances where the uniform silica coating is obtained on a Fe_3O_4 core particles without calcination. The reason for the same is (a) Use of ultra-sonication instead of mechanical stirring for mixing of particles. (b) Controlling the rate of TEOS hydrolysis by using mild conditions for hydrolysis. It was reported in literature that this kind of slow hydrolysis often results in non-Fe_3O_4, only silica nanoparticles, but we didn't notice any such effects.

Synthesis of 5-amino-3-methylisoxazole by using Fe_3O_4@SiO_2: Hydroxylamine hydrochloride, 3-amino crotonitrile and Fe_3O_4@SiO_2 particles are taken in water and the reaction temperature is maintained at 0-25 °C. A mechanical stirrer is used for uniform mixing and the reaction was continued for 6 h and the Fe_3O_4@SiO_2 particles were separated by a magnet. The reaction mixture was basified and filtered. The caution need to be taken to maintain pH around 10 and the filtrate is saved for second crop. The precipitated solid contains most of the product and is suspended in diethyl ether and organic solution is filtered. The ether layer is mixed with the saved mother liquor, organic layer is separated, dried over $MgSO_4$ and concentrated under vacuum around 30 °C. It is observed that upto 10 % of product is remained in saved aqueous layer and the final ether wash is critical in obtaining final yield close to 80 %. The Fe_3O_4@SiO_2 particles can be washed with boiler water and reused.

Reaction yield improvement of 5-amino-3-methylisoxazole: We could obtain a 30 % yield improvement in reactions by using 40 nm size Fe_3O_4@SiO_2 nanomaterials without altering the reported reaction conditions. We also could observe that the 40nm particles seems to be important and our experiments with 100-120 nm traditional

Fe_3O_4@SiO_2 has resulted only in marginal improvement as shown in Table-1. The improvement in reaction yields with decrease in size of nanoparticles is in line with other published observations.

TABLE-1
REACTION YIELDS OF 5-AMINO-3-METHYLISOXAZOLE USING CONVENTIONAL ORGANIC SYNTHESIS AND BY OUR NANOMATERIAL CATALYST

Scale	Conventional synthesis	40 nm Size MNPs@SiO_2	100 nm Size MNPs@SiO_2
1 g	43 ± 2 (n = 2)	72 ± 4 (n = 2)	54 ± 2 (n = 2)
10 g	47 ± 3 (n = 2)	74 ± 3 (n = 3)	52 ± 2 (n = 2)
100 g	44 ± 2 (n = 2)	80 ± 5 (n = 2)	48 ± 1 (n = 2)

Mechanism of action of Fe_3O_4@SiO_2 particles: Silica surface is a partially acidic surface and rarely reported to aid in isoxazole formation. The main mechanism could be offer of solid support for reaction at the temperature of 0-25 °C and plays a major role in yield improvement. Use of montromalite clay [37], silica [38] and alumina [39] are reported to perform similar role in synthesis of other organic compounds [40,41], especially for nitro compounds [42]. For isoxazole synthesis also similar inert solid support catalysts are supported [43,44] and the reaction mechanism shown in **Scheme-II** suggests that acidic nature of silica surface might have a role in reaction yield improvement.

Conclusion

We report almost a 30 % yield improvement for synthesis of 5-amino-3-methylisoxazole using 40 nm Fe_3O_4@SiO_2 nanoparticles. The inert catalysts could play significant role in reaction yield improvement for pharmaceutically important molecules and the uniqueness of this reported protocol will have significant impact on bulk drug synthesis.

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