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Straightforward chemoselective 4-nitration of 5-aminoisoxazoles

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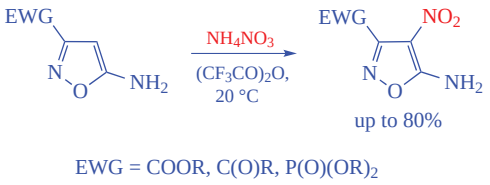
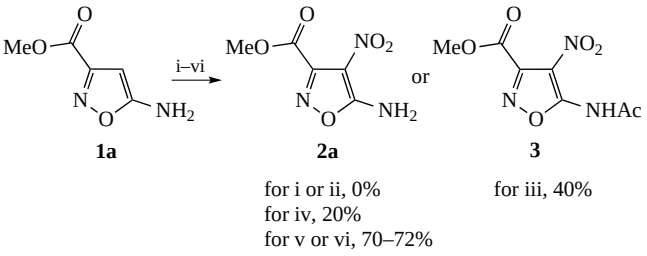
5-Aminoisoxazoles bearing 3-positioned electron-withdrawing group react chemoselectively with trifluoroacetyl nitrate (generated from ammonium nitrate and trifluoroacetic anhydride) to give the corresponding 4-nitro derivatives in yields up to 80%. The results were rationalized by computation studies. Several 5-amino-4-nitroisoxazole-3-carboxilic acid derivatives revealed moderate antibacterial activity.



Keywords: isoxazoles, aminoisoxazoles, nitroisoxazoles, nitration, chemoselectivity, trifluoroacetyl nitrate, quantum chemical calculations, antibacterial activity.

Isoxazole ring is a well-known scaffold for the development of new drug candidates.[1] Isoxazole moiety is present in natural psychoactive molecules such as AMPA, muscimol and ibotenic acid. Isoxazole derivatives are known as marketed antibiotics,[2] anticonvulsants,[3] antipsychotics,[4] antidepressants,[5] anti-inflammatory[6] and antirheumatic[7] drugs. The presence of functional groups at isoxazole ring provides the variability of structural modification of isoxazole compounds, however direct C–H functionalization of this heterocycle still remains problematic.[8] Recently,[9,10] we synthesized poorly accessible 3-EWG-5-nitroisoxazoles (EWG is electron-withdrawing group) basing on heterocyclization of electrophilic alkenes upon the treatment with tetranitromethane (TNM) activated by triethylamine. Also, a novel two-step protocol for the synthesis of 3-EWG-5-aminoisoxazoles from readily available electrophilic alkenes was elaborated.[11] These methods permit obtaining 4-alkyl or 4-unsubstituted isoxazole derivatives.

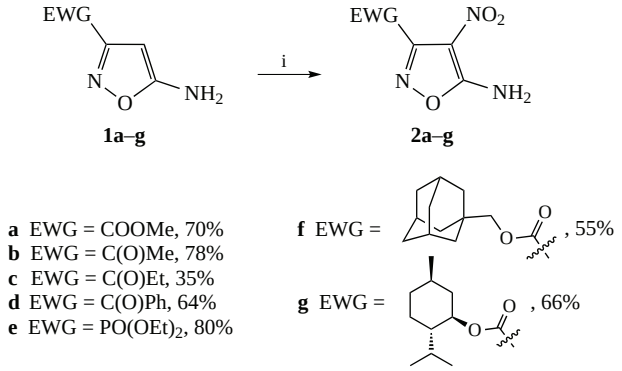
In the present work, direct nitration of isoxazole ring at the unsubstituted position 4 of 3-EWG-5-aminoisoxazoles was studied for the purpose of obtaining isoxazoles bearing functional groups at each carbon atom. In general, nitration of isoxazoles seems to be the easiest approach to 4-nitro substituted heterocycles, however this method has limitations related to the nucleophilicity of the heterocyclic ring and the tolerance of substituents under the nitration conditions.[8,12] Therefore, we tested various nitration systems with 5-aminoisoxazole **1a** as a model substrate (Scheme 1). The known rare examples for the synthesis of amino(nitro)isoxazoles comprised nitration at position 4 with preliminary acylation of the amino group.[12] Meanwhile, due to the electron-withdrawing nature of isoxazole ring low nucleophilicity of the amino group may be anticipated. Hence, we supposed that straighforward nitration of N-unprotected 5-aminoisoxazole **1a** could be possible.



Scheme 1 *Reagents and conditions*: i, HNO_3/Ac_2O; ii, $Ce(NO_3)_3/Ac_2O$; iii, $(NH_4)_2Ce(NO_3)_6/Ac_2O$; iv, NO_2BF_4/MeCN; v, $NH_4NO_3/(CF_3CO)_2O$; vi, $Me_4NNO_3/(CF_3CO)_2O$; room temperature in all cases.

To find the optimal reaction conditions, a variety of mild nitration systems were assessed at room temperature (see Scheme 1 and Online Supplementary Materials, Table S1). The use of acetyl nitrate generated *in situ* from acetic anhydride and nitric acid, cerium nitrate or cerium ammonium nitrate afforded no 5-amino-4-nitro-isoxazole **2a**. The treatment of **1a** with cerium ammonium nitrate in acetic anhydride yielded its acylated derivative **3** as a sole product. When NO_2BF_4 or quaternary ammonium nitrates in trifluoroacetic anhydride were tested, target isoxazole **2a** was obtained in 20–72% yields. The best results (70–72% isolated yields) were achieved when ammonium nitrates in trifluoroacetic anhydride as a solvent were applied.[†] Attempts to use trifluoroacetic anhydride in a mixture with another solvent led to the decrease of yield (see Table S1).

[†] Probably, when trifluoroacetic anhydride is employed as a solvent, the tandem acylation–nitration reaction would proceed, however due to an easy cleavage of trifluoroacetic protection under mild basic conditions,[13] only compound **2a** is isolated.



Scheme 2 *Reagents and conditions*: i, $NH_4NO_3/(CF_3CO)_2O$, room temperature, 12 h.

With the optimized reaction conditions in hand [NH_4NO_3/$(CF_3CO)_2O$], the substrate scope for the nitration was investigated (Scheme 2). Substrates **1a**–**g** were converted into products **2a**–**g** in good yields.‡

Lower yield of 5-amino-4-nitroisoxazole **2c** (see Scheme 2) can be explained by poor solubility of the starting compound **1c** in trifluoroacetic anhydride. The structure of 5-amino-4-nitroisoxazoles was confirmed by X-ray crystal structure analysis on the example of compound **2f** (Figure 1).§

To gain a deeper understanding of the reactivity of 5-substituted izoxazoles in the course of nitration, we performed quantum-chemical simulations for 3-methoxycarbonyl-containing 5-aminoisoxazole **1a** in comparison with 5-nitroisoxazole **4** (Figure 2). The FMO plots show that the HOMO of 5-amino derivative **1a** has large molecular orbital coefficient at C^4 atom that makes it reactive towards electrophiles.

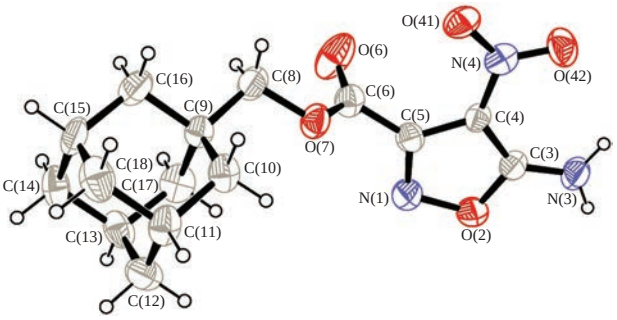


Figure 1 The molecular structure of **2f**, showing the atom-numbering scheme. Displacement ellipsoids are drawn at the 50% probability level, H atoms presented as spheres with arbitrary radius.

On the other hand, the HOMO of 5-nitro analogue **4** is mainly localized in carboxyl group, while for C^4 and C^5 of isoxazole ring small molecular orbital coefficients were calculated. Computational details and energies are given in Online Supplementary Materials.

Indeed, when trying to perform 4-nitration of 5-nitroisoxazole **4** under the optimal conditions for 5-aminoisoxazoles [$NH_4NO_3/(CF_3CO)_2O$] or upon the treatment with HNO_3/Ac_2O, H_2SO_4/HNO_3, or oleum–HNO_3 at room temperature or at 120 °C, only the starting compound was recovered. Thus, the computation is in a good agreement with the reactivity of isoxazole derivatives towards nitration reagents.

For 5-amino-4-nitroisoxazoles **2a**–**g** and acetylated analogue **3**, primary screening of antibacterial activity against Gram positive bacteria (*Bacillus subtilis* ATCC 6633) and Gram negative bacteria (*Escherichia coli* ATCC 25922), as well as antifungal activities against two strains (*Aspergillus niger* INA 00760 and *Candida albicans* ATCC 2091*)* was performed by disc diffusion assay. Compounds **2d**,**g** and **3** suppressed microbial growth of Gram-positive bacteria (*Bacillus subtilis* ATCC 6633) (**2g**, **3**) and Gram-negative bacteria (**2d**, **3**). Compound **3** was found to demonstrate moderate antifungal activity against *Aspergillus niger* INA00760 test strain. For active compounds **2d**,**g** and **3** we also studied antibacterial activity against methicillin-resistant *Staphylococcus aureus* strain (MRSA). All tested compounds exhibited moderate antibacterial activity with the best results for 4-nitroisoxazole **3** bearing acylated amino group (see Online Supplementary Materials, Table S3).

In conclusion, simple, mild and effective procedure for the synthesis of 5-amino-4-nitroisoxazoles with variety of functional groups in position 3 was elaborated. This method allows one to carry out the nitration of isoxazoles with unprotected amino group avoiding the additional steps of protection–deprotection sequence. A novel series of polyfunctionalized heterocycles were synthesized and their antibacterial and antifungal activities were estimated. Some of the tested compounds exhibited

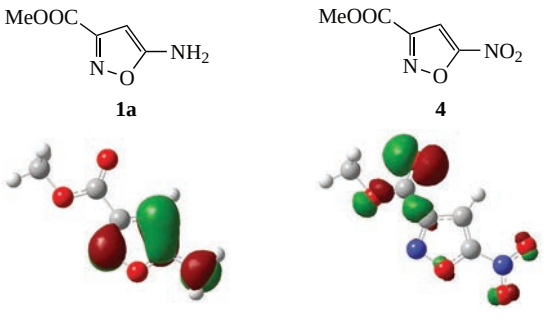


Figure 2 HOMOs of 3-methoxycarbonyl-containing 5-aminoisoxazole **1a** and 5-nitroisoxazole **4**. FMOs were generated at the B3LYP/6-31G(d,p) level.

‡ *General procedure for the synthesis of 5-amino-4-nitroisoxazoles* **2a**–**g**. Ammonium nitrate (20 mg, 0.25 mmol) was added to a solution of 5-aminoisoxazole (0.25 mmol) in $(CF_3CO)_2O$ (2 ml). The mixture was stirred for 12 h and then poured into ice water and neutralized with $NaHCO_3$ (aq.) to reach pH 7–8. The product was extracted with CH_2Cl_2 (4 × 10 ml). The combined organic extracts were dried over $MgSO_4$, the solvent was removed under reduced pressure, and the product was isolated by column chromatography.

Methyl 5-amino-4-nitroisoxazole-3-carboxylate **2a**. Colourless solid, yield 33 mg (70%), mp 141 °C, R_f 0.56 (light petroleum/EtOAc, 2 : 1). ^{1}H NMR ($CDCl_3$–CD_3OD) δ: 3.95 (s, 3H, Me). The signal of NH_2 group was eclipsed by the signals of solvent. ^{13}C NMR ($CDCl_3$–CD_3OD) δ: 53.9 (OMe), 109.1 (CNO_2), 151.2 (C), 159.1 (C), 166.3 (C). HRMS (ESI), *m*/*z*: 188.0305 (calc. for $C_5H_6N_3O_5^+$, *m*/*z*: 188.0302 $[M+H]^+$).

§ *Crystal data for* **2f**. $C_{15}H_{19}N_3O_5$ (M = 321.33), monoclinic, space group $P2_1/c$, at 295(2) K: a = 20.3316(16), b = 6.7361(4) and c = 11.5636(9) Å, α = 90°, β = 100.899(6)°, γ = 90°, V = 1555.1(2) Å^3, Z = 4, d_{calc} = 1.372 g cm^{-3}, μ = 0.875 mm^{-1}, F(000) = 680. Total of 6897 reflections were collected [2889 independent reflections, R_{int} = 0.0715 (before absorption correction)] and used in the refinement, which converged to wR_2 = 0.0687, GOOF 0.630 for all independent reflections [R_1 = 0.0529 was calculated for 2889 reflections with $I > 2\sigma(I)$].

Single crystals suitable for X-ray diffraction were obtained by slow evaporation of the solvent from a toluene solution of **2f**. Intensity data were collected on a Stoe STADI VARI diffractometer using focusing mirrors monochromated CuKα radiation, λ = 1.54186 Å. The data were corrected for decay, Lorentz, and polarization effects as well as absorption and beam corrections based on the multi-scan technique. The structure was solved by a combination of direct methods in SHELXS-97 and the difference Fourier technique, and refined by full-matrix least-squares procedures (SHELXL-2015). Non hydrogen atoms were refined with anisotropic displacement parameters. The H-atoms were calculated and subsequently treated with a riding model.

CCDC 984431 contains the supplementary crystallographic for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre *via* http://www.ccdc.cam.ac.uk.

moderate antibacterial activity against Gram positive and Gram negative bacteria that opens the possibility of further optimization of the structure in order to find a lead compound for the development of the novel antibacterial drugs.

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Online Supplementary Materials

Supplementary data associated with this article can be found in the online version at doi: 10.1016/j.mencom.2020.07.027.

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