Preparation of *N*-Formamidinylamino Acids from Amino and Formamidinesulfinic Acids

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Abstract: A practical synthetic procedure for the conversion of amino acids into *N*-formamidinylamino acids using formamidinesulfinic acid in basic water solution is presented.

Key words: guanidino acids, guanylation, *N*-formamidinylamine

There is considerable interest for *N*-formamidinylamino acid derivatives due to their exceptionally diverse biological activity. Some areas of research concerned with the biological activities of these compounds are in the fields of the human cerebral arteries,[1] neuronal nitric oxide syntheses,[2,3] inhibition of spontaneous mammary tumors,[4] resistance of the influenza viruses,[5] and anticancer research.[6] Common precursors for preparation of these compounds are amino acids and cyanamide. This approach was originally introduced by Strecker[7] and is probably the most versatile and most reliable procedure for the preparation of non-substituted *N*-formamidinylamines in general. Armstrong later improved this method.[8] Weiss and Krommer[9] successfully used this approach for the preparation of creatine on an industrial scale.[10] For the preparation other *N*-formamidine derivatives other than creatine, this reaction is rather slow. To achieve a high yield, stirring of the reaction mixture at room temperature for several days is required. Other popular methods are reactions of amines with substituted *S*-methylisothiourea,[11] or reactions of amines with 2,5-dimethyl-1-guanylpyrazole.[12] The last two methods are generally better than the cyanamide method, but the reactions usually require a strong basic media and/or heat when carried out using amino acids.[13] There are several reports that use *S*-oxides of thiourea for the preparation of *N*-formamidinylamines.[14,15] In their studies, Miller and Bischoff used formamidesulfonic acid [$HN{=}C(NH_2)SO_3H$] and some *N*-phenyl derivatives of this acid to prepare a few *N*-formamidinylamino acids in 25–85%.[15] In theory, a more accessible formamidinesulfinic acid [$HN{=}C(NH_2)SO_2H$] should also be useful as an *N*-formamidinylation reagent. According to Miller and Bischoff, *N*-formamidinylation of amino acids with this reagent is unreliable and the reaction conversion is negligible.[15] Actually, the first report of *N*-formamidinesulfinic acid as an *N*-formamidinylation reagent was reported by Walter.[16] With this reagent, *N*-formamidinylglycine was prepared from glycine in ammonia in 34% yield.[16] Considering the fact that valuable *N*-formamidinyl amino acid derivatives can be prepared with this reagent, we have decided to explore its *N*-formamidinylation capability with amino acids.

To achieve our target, we have explored *N*-formamidinylation reactions in aqueous ammonia. Both starting materials (glycine and formamidinesulfinic acid) are soluble in aqueous ammonia, and after mixing these reagents, white crystals were formed and a small amount of the product (~10%) was obtained. In addition, very poor yields of other products of amino acids were obtained in ammonia. Not only was a small portion of the amino acid transformed into the *N*-formamidinylamino acid, but also separation of the product from the amino acid and the inorganic salt formed in the course of the reaction is not an easy task.

If the same reaction is performed at room temperature with an equivalent amount of aqueous sodium carbonate, the reaction conversion is still very low. Changing the introduction order of the reactants can substantially increase the reaction conversion. Equivalent amounts of amino acid and sodium carbonate were dissolved in water at an elevated temperature and into this mixture (cooled to room temperature) in small portions over a prolonged period, the formamidine acid was added. The reaction mixture was stirred at room temperature for several hours and then at 60 °C for a half an hour. The *N*-formamidinylation reaction was now performed with ~60% conversion, as determined by comparison of the ^1H NMR signals for hydrogen atoms attached to the chiral carbons of the amino acid and the product.[17] There are a few interesting observations that suggest that this method can be of preparative use. First, the reaction should be performed in very concentrated water media. In many cases, during the course of the reaction, a white precipitate is formed and if the reaction mixture is too concentrated then its proper stirring is hampered. Mechanical stirring of the reaction mixture is advised instead of the more convenient magnetic stirring. The best results are obtained when 0.1 mol of the amino acid is dissolved in 30 mL of 0.1 mol aqueous sodium carbonate. Even in this case, the highest reaction conversion was around 75%, with an isolation yield quite lower than that due to difficulty in separating the product from the starting amino acid and the inorganic salt.

One of the ways to eliminate the starting amino acid from the reaction mixture is to assure its quantitative conversion into the product. Therefore, we gradually increased the amount of base and formamidinesulfinic acid. Indeed,

^{1}H NMR signals for the starting amino acid were not present or were substantially diminished, but with the 40% excess of formamidinesulfinic acid, a new byproduct, cyanoguanidine [HN=C(NH_2)NHCN], was formed.[18] Although cyanoguanidine can be eliminated from the product by several crystallizations, the isolated yield after several crystallizations is still around 30−40%.

In further optimization of the reaction conditions, the base, sodium carbonate, was replaced with sodium hydroxide (2.3 equivalents) and the formamidinesulfinic acid molar ratio was increased to 1.13 equivalents in regards to the amino acid. The amino acid/water ratio was also increased to a 0.1 mol/40 mL ratio to insure better stirring of the highly viscous reaction suspension. Under these reaction conditions, almost quantitative amino acid conversion into product was observed in the crude ^{1}H NMR spectra. Furthermore, a minimal amount of the cyanoguanidine was formed.

As in previous cases, separation of the product from small quantities of starting amino acid and cyanoguanidine, as well as the formed inorganic salt was not an easy task. Most of *N*-formamidinylamino acids are soluble in water, therefore their separation from the inorganic salt by crystallization is an almost impossible task. On the other hand, amino acids with sizeable organic parts such as phenylalanine, phenylglycine, and 5-aminopentanoic acid produce a less water soluble product and can be separated from salt by crystallization from water. It is important to perform the first crystallization with a minimal amount of water. After the first crystallization, the solubility of the product in water is substantially diminished. The other byproduct, cyanoguanidine can be removed by washing the crystalline product with methanol (Procedure A).

If the product of the reaction is substantially soluble in water, then the isolation method presented in procedure A is not appropriate. In these cases, a dry work-up of the reaction mixture was necessary (Procedure B). After the reaction was completed, the water was evaporated at almost room temperature and a white solid was extracted with hot methanol. With this approach, the yield of *N*-formamidinylamino acid is considerably higher. The yields of products generated with these two methods are listed in Table. Unfortunately, this method is not applicable to low-basic anilines such as 4-aminobenzoic acid. In this particular case, only 5−10% reaction conversion was observed. Therefore, we believe that this approach should not be practical for slightly nucleophilic amines, such as aniline derivatives but it should be the method of choice for conversion of aliphatic amines into the corresponding *N*-formamidinyl derivatives.

Mps were determined on an Electrothermal IA 9000 Digital Melting Point Apparatus. Mass Spectroscopy was performed on a Micromass Quattro 2 Triple Quadrapole Mess Spectrometer. The ^{1}H and ^{13}C NMR spectra were recorded on a Varian Gemini NMR spectrometer (400 MHz) with D_2O or D_2O/DMSO-d_6 as solvent.

***D,L*-*N*-Formamidinephenylalanine; Typical Procedure A**

Into aqueous NaOH (40 mL, 9.2 g, 0.23 mol) and *D,L*-phenylalanine (16.5 g, 0.1 mol), formamidinesulfinic acid (12.4 g, 0.115 mol) was added in small portions over 30 min. After approx. 1/3 of formamidinesulfinic acid was added, the reaction mixture became a viscous suspension. The suspension was stirred at r.t. overnight and then heated to 60 °C and stirred for an additional 30 min. The crys-

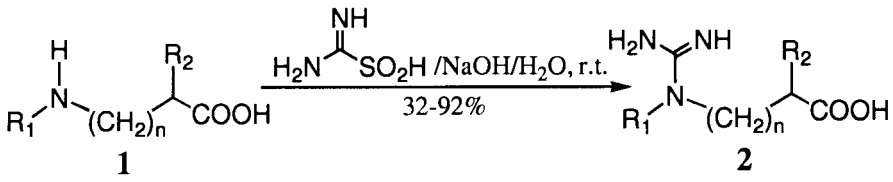

Table Yields of *N*-Foramidine Amino Acids **2**

Entry	Method	Amino Acid **1**	*N*-Formamidine **2**[a]	Yield (%)[b]
2	B	Glycine	*N*-Formamidineglycine	70.2
2	A	Glycylglycine	*N*-Formamidineglycylglicine	70.2
3	B	*N*-Methylglycine	*N*-Formamidine-*N*-methylglycine	83.2
4	B	*L*-Alanine	*N*-Formamidine-*L*-alanine	73.2
5	B	β-Alanine	*N*-Formamidine-β-alanine	72.1
6	A	4-Aminopentane	*N*-Formamidine-β-alanine-4-aminopentane	78.2
7	B	*L*-Tryptophan	*N*-Formamidine-β-*L*-tryptophan	91.8
8	A	*D,L*-Phenylalanine	*N*-Formamidine-*D,L*-phenylalanine	78.2
9	A	*L*-Phenylglycine	*N*-Formamidine-*L*-phenylglycine	82.4
10	B	*L*-Valine	*N*-Formamidine-*L*-valine	32.0
11	A	*L*-Leucine	*N*-Formamidine-*L*-leucine	63.2
12	B	*L*-Methionine	*N*-Formamidine-*L*-methionine	39.3

[a]All products were characterized by comparison of their mps, IR and ^{1}H NMR spectra from references 10 and 16.
[b]Isolated yields.

talline product was separated by cold filtration. White solid was crystallized from H_2O, and the resulting white crystals were washed with small portions of cold MeOH.

Yield: 16.2 g (78.2%), mp: ~235 °C (dec.).

^{1}H NMR (400 MHz, D_2O): δ = 7.28 (dt, 2H, *J* = 0.018, 0.003 Hz), 7.27 (dt, 1H, *J* = 0.018, 0.08 Hz), 7.18 (dd, 2H, *J* = 0.019, 0.008 Hz), 4.10 (dd, 1H, *J* = 0.019, 0.011 Hz), 3.15 (dd, 1H, *J* = 0.035, 0.011 Hz), 2.90 (dd, 1H, *J* = 0.035, 0.020 Hz).

^{13}C (100MHz, D_2O/DMSO-d_6/NaOH): δ = 180, 160, 139, 130, 129,128, 59, 39.

MS (electrospray, ES^+, CH_3OH/H_2O): *m/z* = 188.7, 208.6, 213.6, 230.6 $(M+Na)^+$, 231.7, 232.5.

***L*-*N*-Formamidinetryptophan; Typical Procedure B**

NaOH (9.2 g, 0.23 mol) was dissolved in H_2O (40 mL) and *L*-tryptophan (20.4 g, 0.1 mol) was added. Into the clear reaction mixture at r.t. and with vigorous stirring, small portions (1 g/3 min) of formamidinesulfinic acid (12.4 g, 0.115 mol) was added. After all formamidinesulfinic acid was added, a very hard to stir reaction suspension was agitated at r.t. overnight. Into the suspension, EtOH (100 mL) and toluene (300 mL) were added and these solvents were evaporated at ~30 °C under reduced pressure. The same procedure was repeated two more times to eliminate traces of H_2O. The solid residue was slurred into MeOH (1 L) and refluxed for several hours. The liquid was separated from the solid inorganic material by hot filtration and concentrated into a smaller volume (100 mL). The product was crystallized from MeOH by standing at r.t. for several days. Resulting white crystals were separated by filtration.

Yield: 22.6 g (91.8%), mp: ~225 °C (dec.).

^{1}H NMR (400 MHz, D_2O): δ = 10.84 (s, 1H), 7.49 (d, 1H, *J* = 0.021 Hz), 7.32 (br s, 1H), 7.29 (d, 1H, *J* = 0.021 Hz), 7.06 (d, 1H, *J* = 0.005 Hz), 7.01 (dt, 1H, *J* = 0.017, 0.003 Hz), 6.92 (dt, 1H, *J* = 0.019, 0.002 Hz), 4.05 (dd, 1H, *J* = 0.037, 0.019 Hz), 3.21 (dd, 1H, *J* = 0.026, 0.011 Hz).

^{13}C NMR (100 MHz, D_2O/DMSO-d_6/NaOH): δ = 178, 159, 137, 128, 124, 122, 120, 119, 112.7, 112, 58, 29.

MS (electrospray, ES^+, CH_3OH/H_2O): *m/z* = 129.9, 147.8, 178.8, 211.7, 214.8, 229.7, 230.8, 247.7, 251.6, 269.5 $(M+Na)^+$, 270.5, 271.5

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(17) For the *N*-formadinylamino acid, the ^{1}H NMR chemical shift for hydrogen attached to chiral carbon is ~0.2 ppm higher than that of the amino acid.
(18) Byproduct, $HN=C(NH_2)NHCN$ is probably formed by cyanamide dimerization after the (Na_2SO_2) is eliminated in sodium hydroxide water solution of formamidinesulfinic acid.

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