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Synthesis and inhibitory activity of benzoic acid and pyridine derivatives on influenza neuraminidase

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Abstract—Based upon the activity and X-ray crystallographic studies of tri-substituted benzene derivatives containing carboxylic acid, acetamido and guanidine groups, we investigated the effect of the fourth substituent to fulfill the fourth pocket of neuraminidase enzyme. The groups selected as fourth substituents were hydroxymethyl, hydroxyethyl, oxime and amino. These tetra-substituted benzene derivatives were synthesized and evaluated for neuraminidase inhibitory activity. All these compounds were found to have poorer IC_{50} values than the tri-substituted compounds. Further, benzene ring was replaced by pyridine ring and di, tri and tetra-substituted pyridine derivatives were synthesized. The activity of the pyridine derivatives was comparable to benzene derivatives. The fourth substituent seems to disturb the binding of the other three substituents, so the activity is reduced as compared to tri-substituted benzene and pyridine derivatives.

1. Introduction

Influenza commonly known as flu is a major cause for health concern and economic costs.[1,2] Approximately 120 million people in North America, Europe and Japan are infected each year, and about 10,000–40,000 deaths occur annually in the US primarily among the elders and people with suppressed immune systems. The pandemic of 1918–1919 has been responsible for the death of 20 million people.[3] Since then, less severe pandemics have occurred every 10–20 yr, resulting in a new virulent strain contributing from the viral ability to modify its surface antigens. The viral replicative cycles involve two essential surface glycoproteins, hemagglutin and sialidase (neuraminidase). These proteins are present on the surface of the influenza virus. Hemagglutin is important for the infection process and neuraminidase is required for the release of newly formed virus. Inhibition of either surface glycoproteins by complexation with active sites would lead to drugs, which could be used to treat infection arising from flu virus.[4–6]

Until the approval in 1999 of new agents, amantadine and rimantadine were the only antiviral agents used for influenza therapy. These drugs are of limited usefulness because they lack activity against influenza virus B and cause unwanted side effects.[7] Analogues of neuraminic acid, such as 2,3-didehydro-2-deoxy-N-acetylneuraminic acid (**1**, DANA, Chart 1) are known to inhibit neuraminidase in vitro with a K_i value of 0.004 mM.[8] The replacement of hydroxyl group in DANA with a guanidino group resulted into a FDA approved drug, zanamivir (**2**, Chart 1), which is a potent inhibitor of influenza A and B.[9–11] It was approved by FDA in 1999 and is used as an oral inhaler, which may be inconvenient for the children and elderly people. Another compound, oseltamivir carboxylate (**3b**, Chart 1; active compound is **3a**, Chart 1) was also approved by FDA in 1999 as an oral drug.[12] This compound also is very effective for both influenza A and B, but has been reported to have some side effects. One compound, BCX-1812 (RWJ-270201, **4**, Chart 1), which is equally effective in vitro[13–15] and in vivo[16–19] on influenza A and B from our laboratories also reached to clinical phase-III trials but did not show statistical difference in efficacy probably because of lower bioavailability. Some other modifications of BCX-1812 were also made but those changes did not result into a oral drug candidate.[20] However, all these reported molecules have

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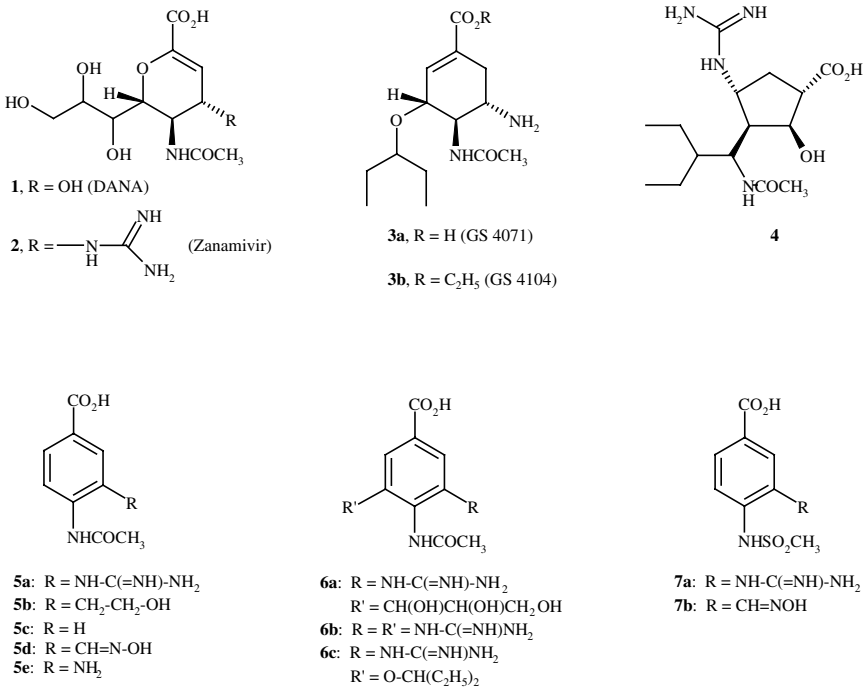


Chart 1.

shown the importance of all four substituents (carboxylic, acetamido, guanidine or amine and glycerol or hydrophobic) for the activity on neuraminidase A and B. The discovery of cyclopentane by us and pyrrolidine by Abbott laboratories[21–24] also revealed that it is not necessary to have a six membered cyclohexyl or pyranose ring.

Some time back, our laboratories successfully designed aromatic compounds for this project and were able to get a compound BCX-140 (**5a**, Chart 1), having IC_{50} value of 0.0025 mM, which is very appropriate for a compound having only three substituents (carboxyl, acetamido and guanidine) on the benzene ring.[25] Meanwhile, more work was done by us and other laboratories and reported the compounds having fourth substituent as glycerol (**6a**, Chart 1, $IC_{50} = > 0.1$ mM),[26] guanidine (**6b**, Chart 1, $IC_{50} = 0.01$ mM)[27] and hydrophobic (**6c**, Chart 1, $IC_{50} = 0.003$ mM).[28] The inhibitory activity of all these compounds for neuraminidase is not better than BCX-140. It is apparent from these values that the fourth substituent is not only pointing to the wrong direction but also disturbing the positions of other groups to interact with the amino acid residues in the active site. Therefore, we decided to investigate some other groups as the fourth substituent on benzene ring and also changing the benzene ring by a pyridine ring. Herein we report the synthesis and neuraminidase inhibitory activity of tetra-substituted benzene derivatives and also of some di-, tri- and tetra-substituted pyridine analogs.

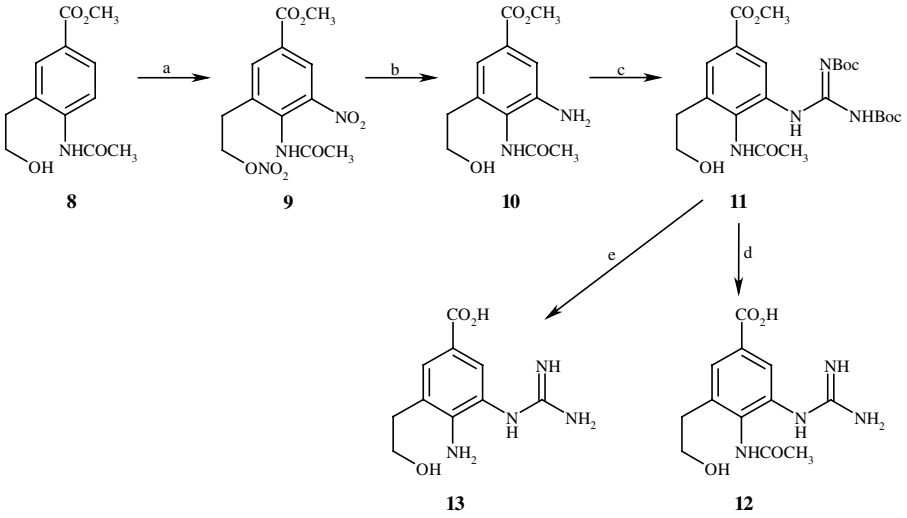
2. Chemistry

The reported compound **8**[25] (Scheme 1) on nitration using fuming HNO_3 gave **9**, which on catalytic hydrogenation gave amino compound **10**. The guanidine group was introduced on the amino group of **10** using N,N′-bis(*tert*-butoxycarbonyl)thiourea (bisBocthiourea) in the presence of $HgCl_2$ and triethylamine to furnish **11**. Deprotection of Boc groups using trifluoroacetic acid, followed by base hydrolysis gave target compound **12**. However, hydrolysis of **11** with concd HCl gave deacetylated compound **13**.

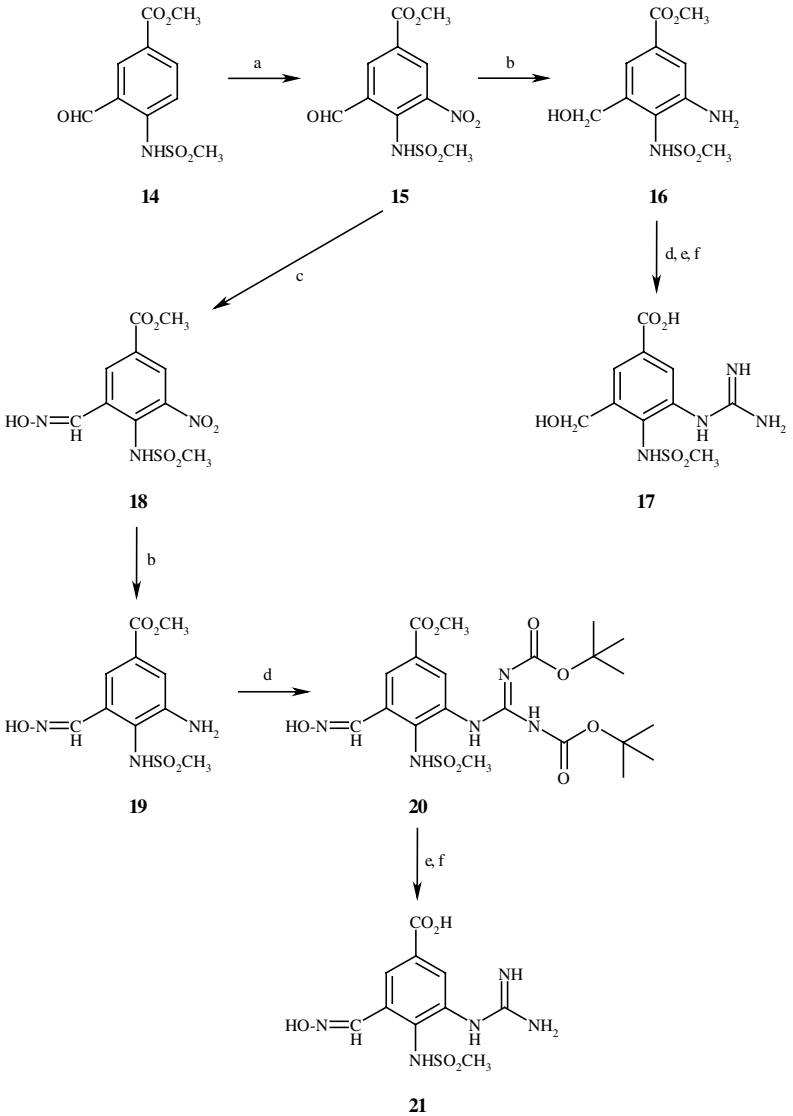
The compound **14**,[25] on nitration with fuming HNO_3 gave nitro derivative **15**. Compound **15** on hydrogenation in the presence of PtO_2 produced **16**, while on reaction with hydroxylamine hydrochloride gave oxime **18**. Oxime **18** on hydrogenation in the presence of PtO_2 produced **19**. The alcohol **16** and oxime **19** were converted to the tetra-substituted targets **17** and **21**, respectively (Scheme 2) by following the sequence of guanylation, Boc deprotection and hydrolysis as in Scheme 1 for target **12**.

The di-substituted compounds of pyridine were prepared starting from the known ethyl-5-aminopyridine-2-carboxylate, compound **22**.[29] Sulfonylation using methane sulfonyl chloride and acetylation using acetic anhydride of compound **22** gave compounds **23** and **25**, respectively. Treatment of **25** with Lawesson's reagent gave the corresponding thioamide **27**. Base hydrolysis of compounds **23**, **25** and **27** gave the corresponding di-substituted targets **24**, **26** and **28** of pyridine (Scheme 3).

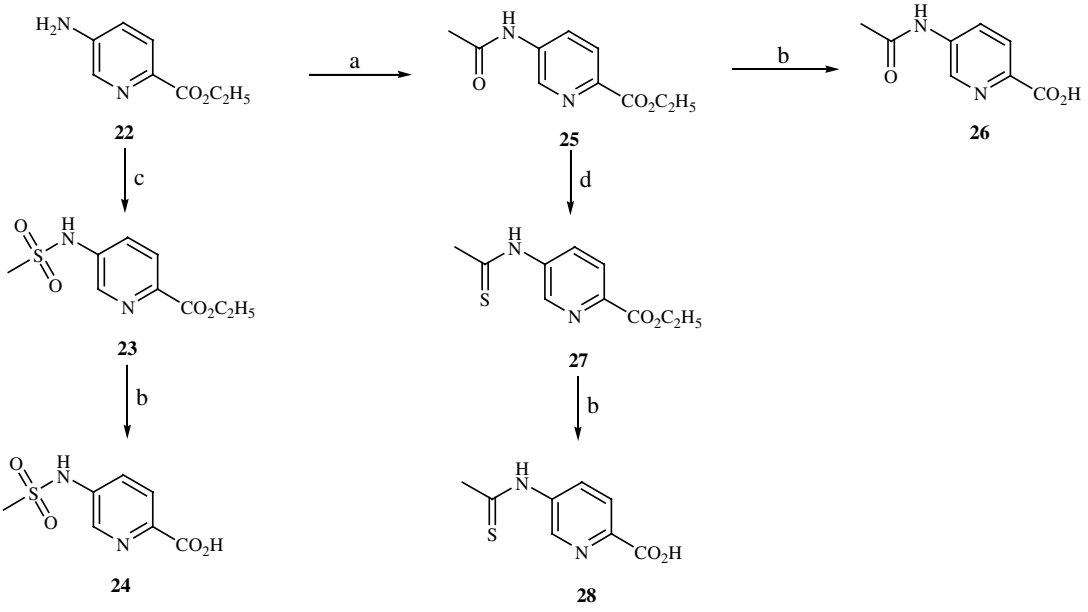
The synthesis of tri-substituted compounds of pyridine (Schemes 4 and 5) starts from reported compounds 2-amino-6-methyl-3-nitropyridine (**29**) and ammonium 4-amino-5-nitropyridine-2-carboxylate (**36**).[30] Compound **31** was obtained via the $KMnO_4$ mediated oxidation fol-



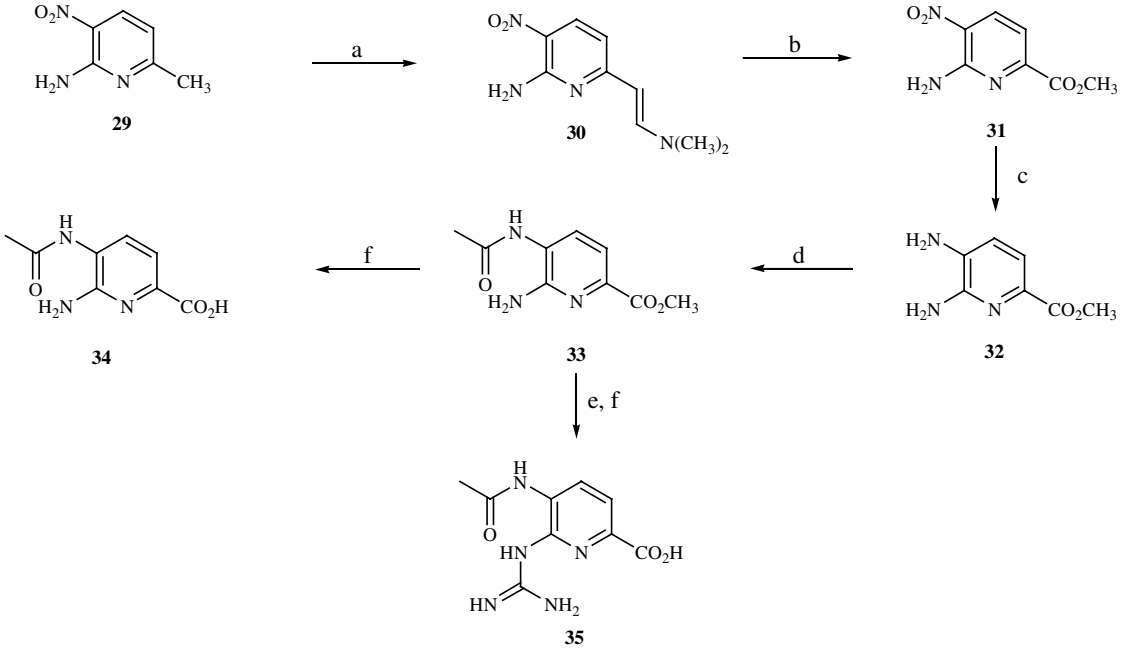
Scheme 1. Reagents and conditions: (a) fuming HNO_3; (b) H_2/PtO_2, MeOH; (c) BocHNC(=S)NHBoc, TEA, $HgCl_2$, DMF; (d) (1) CF_3CO_2H, CH_2Cl_2; (2) NaOH; (3) AcOH; (e) concd HCl.



Scheme 2. Reagents and conditions: (a) fuming HNO_3; (b) PtO_2, H_2; (c) NH_2OH, HCl; (d) BocHNC(=S)NHBoc, TEA, $HgCl_2$, DMF; (e) CF_3CO_2H, CH_2Cl_2; (f) (1) NaOH, (2) $AcOH/H^+$.



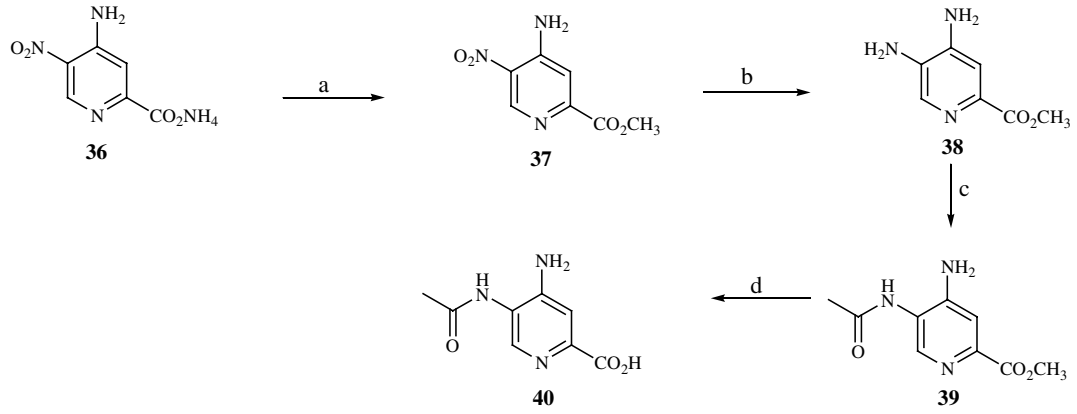
Scheme 3. Reagents and conditions: (a) Ac_2O, AcOH; (b) NaOH, H^+; (c) CH_3SO_2Cl, pyridine; (d) Lawesson's reagent.



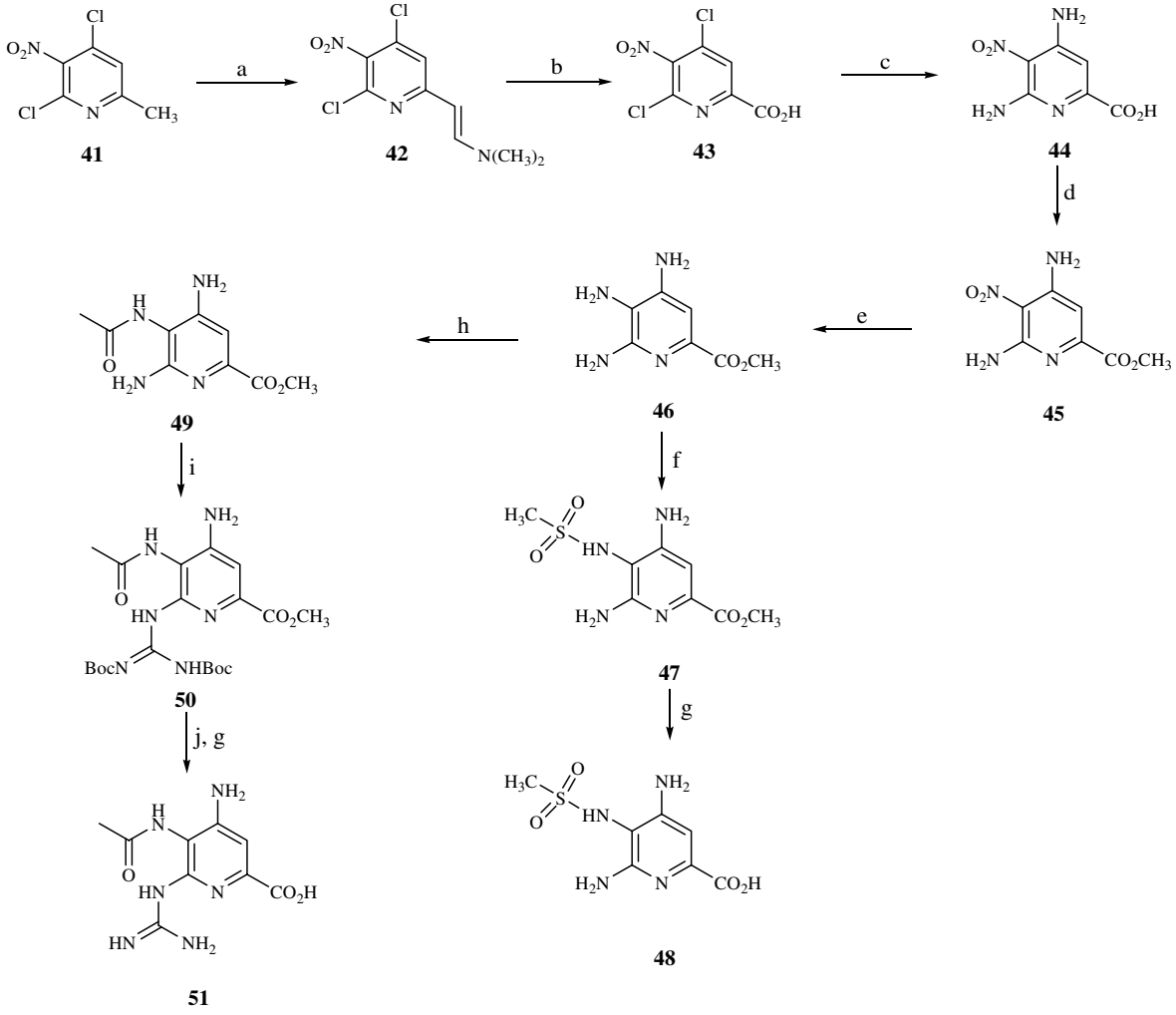
Scheme 4. Reagents and conditions: (a) $(CH_3O)_2CHN(CH_3)_2$, DMF; (b) (1) $KMnO_4$, K_2CO_3, aq *tert*-BuOH; (2) MeOH, H_2SO_4; (c) H_2/PtO_2; (d) Ac_2O, pyridine; (e) H_2NCN, HCl, EtOAc; (f) NaOH, H^+.

lowed by esterification of the imine (**30**) obtained by the reaction of dimethylformamide dimethylacetal with compound **29**. Compound **31** was reduced to the diamino compound **32**. Acetylation was selectively achieved at 3-position of the pyridine ring to furnish compound **33**. Base hydrolysis of **33** furnished target **34**. The guanylation of compound **33** was achieved by using cyanamide under acidic conditions, which followed by base hydrolysis furnished target **35** (Scheme 4). Compound **37** was obtained from compound **36** by refluxing in methanol containing sulfuric acid. Reduction of compound **37** gave diamino compound **38**, which was selectively acetylated at 5-position to furnish compound **39**. Base hydrolysis of **39** furnished the target compound **40** (Scheme 5).

The syntheses of tetra-substituted compound of pyridine (Scheme 6) started from known 2,4-dichloro-3-nitro-6-methylpyridine (**41**).[31] Compound **43** was obtained by the $KMnO_4$ oxidation of imine **42** obtained by the reaction of dimethylformamide dimethylacetal with compound **41**. The dichloro groups were displaced with



Scheme 5. Reagents and conditions: (a) MeOH, H_2SO_4; (b) H_2/PtO_2; (c) Ac_2O, TEA; (d) NaOH, H^+.



Scheme 6. Reagents and conditions: (a) $(CH_3O)_2CHN(CH_3)_2$, DMF; (b) $KMnO_4$, K_2CO_3, aq *tert*-BuOH; (c) NH_3/MeOH; (d) MeOH, H_2SO_4; (e) H_2/PtO_2; (f) CH_3SO_2Cl, pyridine; (g) NaOH, H^+; (h) Ac_2O, pyridine; (i) BocHNC(=S)NHBoc, $HgCl_2$, TEA; (j) CF_3CO_2H, CH_2Cl_2.

amino groups using methanolic ammonia in a bomb to furnish the diamino compound **44**. Esterification of acid in methanol gave ester **45**. The ester was subjected to catalytic reduction to furnish the triamino compound **46**. The 5-amino group in the triamino compound was selectively sulfonylated and acetylated to furnish compounds **47** and **49**, respectively. The 2-amino group of compound **49** was converted to guanidine by the reaction of **49** with bisBocthiourea in the presence of $HgCl_2$ and triethylamine to give **50**. Deprotection of the Boc

group in **50** using trifluoroacetic acid followed by base hydrolysis gave target **51**. Similarly base hydrolysis of compound **47** furnished target **48**.

3. Results and discussion

Compound **5a** (BCX-140) was earlier reported to be the most potent compound in tri-substituted (CO_2H, $NHCOCH_3$ and $NHC(=NH)NH_2$) series of benzene derivatives. Introduction of the fourth group was envisioned to fulfill all the interactions needed for the inhibition of neuraminidase. First we chose CH_2CH_2OH (hydroxyethyl)) group as fourth substituent because, (i) it can serve as hydrophobic group as well as hydrophilic, a combination of glycerol side chain (xanamivir) and alkyl (tamiflu); (ii) X-ray crystallographic studies with tri-substituted compound (having CO_2H, $NHCOCH_3$ and CH_2CH_2OH) showed that CH_2CH_2OH group goes in the pocket where glycerol side chain of xanamivir goes (Fig. 1), while in compound **5a**, guanidine group also goes into the pocket where glycerol side chain of xanamivir goes. We thought that by putting this hydroxyethyl as fourth substituent, guanidine group would go back to the guanidine pocket of xanamivir and hydroxyethyl would stay in glycerol pocket; and (iii) the activity of tri-substituted compound having carboxylic, acetamido and hydroxyethyl (**5b**) was little better as compared to di-substituted compound (**5c**). When the compound **12** was prepared and soaked with the crystals of neuraminidase, guanidine group did go to the guanidine pocket and hydroxyethyl to the glycerol pocket (Fig. 1, showing tri-substituted compounds, **5a**, **5b** and **12**). The result of IC_{50} value (0.4 mM) was disappointing, which can be explained by hydroxyethyl group not pointing to the same direction as glycerol in xanamivir and also disturbed the binding affinities of other groups. During synthesis, acidic conditions of acid hydrolysis provided a deacetylated compound **13**, which was also tested for inhibitory activity, which showed no activity up to 7 mM. This result further shows the importance of acetylamino group.

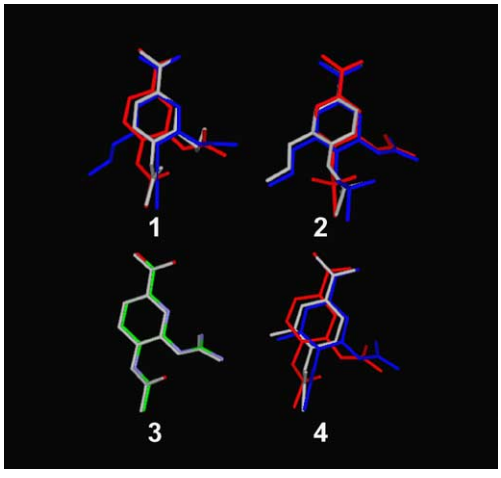


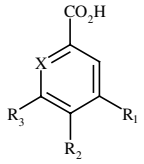
Figure 1. X-ray crystal structure of compounds in the active site of N9 neuraminidase (1) **5a** (red), **5b** (gray) and **12** (blue). (2) **7b** (red), **5d** (gray) and **21** (blue). (3) **5a** (gray) and **35** (green). (4) **5a** (red), **5e** (gray) and **51** (blue).

The next fourth substituent, we chose was CH=NOH (oxime) with $NHSO_2CH_3$ in place of acetylamino. The reasons for this selection were (i) tri-substituted compound having CO_2H, $NHSO_2CH_3$ and oxime (compound **7b**) had better activity (IC_{50} 2 mM) than the corresponding compound with acetylamino, **5d** (IC_{50} 5.5 mM) as shown in the Table 1; (ii) oxime went into the pocket where glycerol side chain of xanamivir goes in the tri-substituted compound (Fig. 1), (iii) oxime group should orient differently than hydroxyethyl. When this compound **21** (with carboxyl, oxime, guanidine and $NHSO_2CH_3$) was prepared and soaked with the crystals of neuraminidase, the binding of the groups was same as expected, guanidine group flipped back to guanidine pocket and oxime to glycerol pocket (Fig. 1 showing compounds **7b**, **5d** and **21**). However, the IC_{50} (0.08 mM) was again not encouraging. A compound **17** with CO_2H, $NHSO_2CH_3$ and CH_2OH and guanidine group was also prepared, which had very poor inhibitory activity. However the results from other laboratories on tetra-substituted compounds were also not encouraging as shown in the Table 1 (Compounds **6a** and **6b**).

Since benzene derivatives were not leading us to a better compound, we explored the pyridine ring system. We started from di-substituted pyridine derivatives (having carboxylic acid and 5-acetylamino, 5-methanesulfonylamino or 5-thioacetylamino) and compared with the corresponding benzene derivatives. The results were very much comparable as shown in Table 1. Although compound **28** with $NHCSCH_3$ is best (IC_{50} 0.4 mM) in all three, but attempts to introduce third substituent with $NHCSCH_3$ failed. Therefore, we selected compound **26** with $NHCOCH_3$ to introduce third substituent. Amino group was introduced in **26** in addition to 5-acetylamino at both positions 4 and 6, separately (compounds **34** and **40**). The IC_{50} values were better than the corresponding benzoic acid derivatives, which encouraged us to convert amino to guanidine (compound **35**). Soak studies were done on compound **35** and compared with corresponding benzene derivative **5a** (Fig. 1 showing compounds **5a** and **35**). Both compounds bound exactly the same. The introduction of fourth substituent, amino in addition to carboxylic, acetylamino and guanidine (compound **51**) gave IC_{50} value of 0.004 mM, which is very comparable to the best tri-substituted compound **5a** (IC_{50} 0.0025 mM), but not better. The comparable binding of tri-substituted benzene derivatives (**5a** and **5e**) and **51** are shown in Figure 1. Another tetra-substituted compound **48** was also prepared, which again was a poor inhibitor.

The best tetra-substituted compound prepared in this paper in benzene series seems to be compound **21** (IC_{50} = 0.08 mM). The addition of a fourth group seems to be decreasing the activity as shown by previous workers also for compound **6a** and **6b**. Compound **6a** (IC_{50} > 0.1 mM) does have all the groups present in GANA to fulfill the interactions with the enzyme and make it a good inhibitor. This decreased potency may

Table 1. In vitro influenza neuraminidase inhibitory activity of aromatic compounds



Cpd.	X	R_1	R_2	R_3	In vitro activity	
					% Inhib. at 7 mM	IC_{50} (mM)
5a	CH	$NHC(=NH)NH_2$	$NHCOCH_3$	H		0.0025
5b	CH	H	$NHCOCH_3$	CH_2CH_2OH	42	
5c	CH	H	$NHCOCH_3$	H	39	
5d	CH	H	$NHCOCH_3$	CH=NOH		5.5
5e	CH	NH_2	$NHCOCH_3$	H	30 at 3.5 mM	
6a	CH	$NHC(=NH)NH_2$	$NHCOCH_3$	$CH(OH)CH(OH)CH_2OH$		>0.1
6b	CH	$NHC(=NH)NH_2$	$NHCOCH_3$	$NHC(=NH)NH_2$		0.01
6c	CH	$NHC(=NH)NH_2$	$O–CH(C_2H_5)_2$	$NHC(=NH)NH_2$		0.003
7a	CH	$NHC(=NH)NH_2$	$NHSO_2CH_3$	H		0.1
7b	CH	H	$NHSO_2CH_3$	CH=N–OH		2
12	CH	$NHC(=NH)NH_2$	$NHCOCH_3$	CH_2CH_2OH		0.4
13	CH	$NHC(=NH)NH_2$	NH_2	CH_2CH_2OH	>7	
17	CH	$NHC(=NH)NH_2$	$NHSO_2CH_3$	CH_2OH		2.0
21	CH	$NHC(=NH)NH_2$	$NHSO_2CH_3$	CH=NOH		0.08
24	N	H	$NHSO_2CH_3$	H		3.0
26	N	H	$NHCOCH_3$	H		2.0
28	N	H	$NHCSCH_3$	H		0.4
34	N	H	$NHCOCH_3$	NH_2		3.0
35	N	H	$NHCOCH_3$	$NHC(=NH)NH_2$		0.006
40	N	NH_2	$NHCOCH_3$	H		0.07
48	N	NH_2	$NHSO_2CH_3$	NH_2		1.0
51	N	NH_2	$NHCOCH_3$	$NHC(=NH)NH_2$		0.004

be because of the way in which the groups are orienting out from a flat aromatic ring. Replacement of benzene ring with pyridine ring gave compound **35** (IC_{50} 0.006 mM) as the most potent compound in tri-substituted series and it mimics compound **5a**. Addition of a fourth group in compound **51** did increase the potency slightly (IC_{50} 0.004 mM) but was not significant. The crystal structure of compound **51** showed that the affinity of guanidine group to glycerol pocket was much more than the guanidine pocket itself. The interaction of pyridine nitrogen in the active sight was not of relevance for the activity.

From the previously reported data and these studies, it appears that six membered aromatic flat structure may not be appropriate core structure for neuraminidase inhibitors, therefore further work may not be necessary on these types of the compounds. Our future plans for this project are to improve the bioavailability of BCX-1812 by making prodrugs, which went up to the clinical trials phase-III.

4. Experimental section

4.1. Enzyme assay

The in vitro assay is based on the method reported by von Itzstein et al.[32] The neuraminidase from the H1N9 strain of influenza was obtained by the method described by Laver et al.[33] Values for the IC_{50} were measured via a spectrofluorometric technique that uses 2′-(4-methylumbelliferyl)-α-D-acetylneuraminic acid as substrate. This substrate was cleaved by neuraminidase to yield a fluorescent product, which can be quantified. The assay mixture contained inhibitors at various concentrations (4–6 points) and enzyme in 32.5 mM MES ((2-(*N*-morpholino)ethanesulfonic acid) buffer, 4 mM $CaCl_2$ at pH 6.5 (total volume = 80 μL). The reaction was started by the addition of 20 μL of the substrate to a final concentration of 75 μM. After 10 min at 37 °C, 2.4 mL of 0.1 M glycine/NaOH (pH 10.2) was added to 0.1 mL of the reaction mixture to terminate the reaction. A blank was run with the same substrate solution with no enzyme. Fluorescence was read using an Aminco-Bowman fluorescence spectrophotometer (excitation, 360 nm, emission, 450 nm), and substrate blanks were subtracted from the sample readings. The IC_{50} was calculated by plotting percent inhibition versus the inhibitor concentration, and determination of each point was performed in duplicate.

4.2. Chemistry. General procedures

Melting points were determined in open capillary tubes in a Mel-Temp II melting point apparatus and are uncorrected; ^1H NMR spectra were obtained in $CDCl_3$, Me_2SO-d_6 or TFA-*d* with Me_4Si as internal standard or in D_2O or ND_4OD with DSS as internal standard on a Bruker AM400 or Bruker AM360 spectrometer; IR spectra were run as KBr pellets on a BioRad FTS-7 FTIR spectrometer; mass spectra were determined on

a Fisons Trio 2000 quadrupole mass spectrometer. Elemental analyses were obtained from Atlantic Microlab, Inc. (Norcross, GA) and are within 0.4% of the calculated values unless otherwise noted.

4.3. β-{[2-(N-Acetylamino)-5-methoxycarbonyl-3-nitro]-phenyl}-O-nitro ethanol (9)

To fuming nitric acid (50 mL, 90%) cooled in an ice bath was added slowly compound **8** (5.0 g, 21 mmol). After the reaction mixture was stirred at 0–5 °C for 45 min and at room temperature for an additional 45 min, it was poured into ice water (100 mL). The light yellow solid obtained was collected by filtration and dried under vacuo to furnish 7.68 g of product. The solid was recrystallized from ethyl acetate to furnish 5.24 g (64%) of **9** as a light yellow, crystalline solid, mp 147–150 °C. ^{1}H NMR (DMSO-d_6): δ 10.21 (s, 1H), 8.25 (d, J = 1.9 Hz, 1H), 8.24 (d, J = 1.9 Hz, 1H), 4.73 (t, J = 6.7 Hz, 2H), 3.90 (s, 3H), 3.23 (t, J = 6.7 Hz, 2H), 2.08 (s, 3H). IR (KBr): 3277, 1731, 1669, 1626, 1535, 1356, 1284 cm^{-1}. MS (ES$^-$), *m/z*: 326.0. Anal. ($C_{12}H_{13}N_3O_8$) C, H, N.

4.4. β-{[2-(N-Acetylamino)-5-methoxycarbonyl-3-amino]-phenyl}ethanol (10)

To compound **9** (3.6 g, 11.0 mmol) in ethanol (200 mL, dissolved with slight heating) was added platinum oxide (0.7 g) under nitrogen. The resulting suspension was hydrogenated for 45 min at 30 psi. To the slurry was added methanol (100 mL) and the catalyst was removed by filtration through Celite. The solvent was removed in vacuo to obtain 2.78 g of white solid, which was recrystallized from ethanol to obtain 2.58 g (93%) of **10** as a light pink solid, mp 186–190 °C. ^{1}H NMR (DMSO-d_6): δ 9.11 (s, 1H), 7.22 (d, J = 1.9 Hz, 1H), 7.06 (d, J = 1.9 Hz, 1H), 5.05 (s, 2H), 4.69 (t, J = 2.1 Hz, 1H), 3.79 (s, 3H), 3.52 (dt, J = 7.1 and 2.1 Hz, 2H), 2.61 (t, J = 7.1 Hz, 2H), 2.05 (s, 3H). IR (KBr): 3514, 3298, 1709, 1642, 1523, 1222 cm^{-1}. MS (ES$^+$), *m/z*: 253.3. Anal. ($C_{12}H_{16}N_2O_4$) C, H, N.

4.5. β-{[2-(N-Acetylamino)-5-methoxycarbonyl-3-[(*t*-butoxycarbonylamino-*t*-butoxy-carbonylimino)methyl]amino]-phenyl}ethanol (11)

To a solution of **10** (2.14 g, 8.0 mmol) in dimethylformamide (8.0 mL) was added triethylamine (4.0 mL, 28.0 mmol) and bisBocthiourea (2.37 g, 8.4 mmol). The resulting mixture was cooled to 0 °C and mercuric chloride (2.39 g, 8.8 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 24 h. Ethyl acetate (65 mL) was added and the slurry filtered through Celite. The filtrate was washed with water (3 × 20 mL), the organic layer dried (Na_2SO_4), filtered and solvent removed in vacuo to give 4.22 g of crude product. The crude product was purified by flash column chromatography (80% ethyl acetate in hexane) to obtain 2.73 g (69%) of **11** as a white solid, mp 120–125 °C. ^{1}H NMR (DMSO-d_6): δ 11.57 (s, 1H), 10.28 (s, 1H), 9.71 (s, 1H), 8.61 (d, J = 1.8 Hz, 1H), 7.70 (d, J = 1.8 Hz, 1H), 4.80 (t, J = 2.1 Hz, 1H), 3.84 (s, 3H), 3.54 (dt, J = 2.1 Hz, 6.3 Hz, 2H), 2.75 (t, J = 6.7 Hz, 2H), 2.10 (s, 3H), 1.52 (s, 9H), 1.41 (s, 9H). IR (KBr): 3249, 2981, 1725, 1644, 1410, 1234 cm^{-1}. MS (ES$^+$), *m/z*: 495.7. Anal. ($C_{23}H_{34}N_4O_8$) C, H, N.

4.6. β-{[2[(N-Acetylamino)-3-[(aminoimino)methyl]amino]-5-carboxyphenyl}ethanol (12)

To a solution of **11** (0.9 g, 1.8 mmol) in methylene chloride (18 mL) at 0 °C was added dropwise trifluoroacetic acid (2.78 mL, 50 mmol) and stirred at room temperature for 16 h. The reaction mixture was filtered and the solvent removed in vacuo. The excess trifluoroacetic acid was removed by co-distilling thrice with methylene chloride (10 mL). To the resulting oily residue, was added water (5 mL) and NaOH (5.4 mL, 1 M, 5.4 mmol) and stirred at room temperature for 1 h. The reaction mixture was filtered and adjusted to pH 7 using glacial acetic acid. The solvent was removed in vacuo to furnish oily residue. Recrystallization of the oily residue twice from water/methanol gave 0.23 g (45%) of **12** as a white solid, mp 224–225 °C (dec). ^{1}H NMR (ND_4OD): δ 8.01 (d, J = 1.9 Hz, 1H), 7.85 (d, J = 1.9 Hz, 1H), 4.24 (t, J = 7.0 Hz, 2H), 3.30 (t, J = 7.0 Hz, 2H), 2.67 (s, 3H). IR (KBr): 3398, 3160, 1638, 1549, 1392, 1046 cm^{-1}. MS (ES$^+$), *m/z*: 280.9. Anal. ($C_{12}H_{16}N_4O_4$) C, H, N.

4.7. β-{[2-(N-Amino)-3-[(aminoimino)methyl]amino]-5-carboxyphenyl}ethanol hydrochloride (13)

To a solution of **11** (2.47 g, 5.0 mmol) in methylene chloride (20 mL) at 0 °C was added dropwise trifluoroacetic acid (3.8 mL, 50 mmol) and stirred at room temperature for 16 h. After 16 h, additional trifluoroacetic acid (3.8 mL, 50 mmol) was added and the reaction stirred for 1 h at room temperature. The reaction mixture was filtered, the solvent removed in vacuo, and the excess trifluoroacetic acid was removed by co-distilling thrice under vacuo with methylene chloride (10 mL). The resulting oily residue was dissolved in 3 M NaOH (8 mL, 24 mmol) and stirred at room temperature for 30 min. The reaction mixture was filtered and adjusted to pH 6 using 1 N HCl. The solvent was removed in vacuo to furnish a brown residue. To 0.1 g of this crude residue in water was added concd HCl (pH < 1) and the solvent was removed in vacuo to give a light brown solid. The solid was dissolved in isopropanol and filtered to remove inorganic impurities, the filtrate was concentrated and residue redissolved in isopropanol to remove traces of inorganic salts. The filtrate was concentrated (~10 mL) and cooled, the crystalline solid obtained was collected by filtration to furnish 0.04 g of **13** as a light brown solid, mp 224–225 °C (dec). ^{1}H NMR (DMSO-d_6): δ 12.28 (br s, 1H), 9.15 (s, 1H), 7.58 (d, J = 2.0 Hz, 1H), 7.40 (d, J = 2.0 Hz, 1H), 7.25 (br s, 4H), 5.77 (br s, 2H), 3.62 (t, J = 6.5 Hz, 2H), 2.70 (t, J = 6.5 Hz, 2H). IR (KBr): 3386, 1694, 1655, 1633, 1392, 1214 cm^{-1}. MS (ES$^+$), *m/z*: 239.2. Anal. ($C_{10}H_{14}N_4O_3$·HCl) C, H, N.

4.8. Methyl 3-formyl-4-[(methylsulfonyl)amino]-5-nitrobenzoate (15)

Compound **14** (3.85 g, 15.0 mmol) was added portionwise to fuming nitric acid (45 mL) at 5–10 °C over

0.25 h. The mixture was further stirred for 1 h at room temperature and then poured into cold water (200 mL). The yellow precipitate was collected by filtration and washed several times with cold water. The cake was recrystallized from ethyl acetate–hexane to give 2.5 g (55.3%) of **15** as light yellow needles, mp 158–160 °C. ^{1}H NMR (DMSO-d_6): δ 10.10 (s, 1H), 8.65 (d, J = 2.1 Hz, 1H), 8.52 (d, J = 2.1 Hz, 1H), 3.94 (s, 3H), 3.21 (s, 3H). IR (KBr): 1729, 1683, 1538, 1297, 1160 cm^{-1}. MS (ES$^-$), *m/z*: 301.0. Anal. ($C_{10}H_{10}N_2O_7S$) C, H, N.

4.9. Methyl 3-amino-5-hydroxymethyl-4-[(methylsulfonyl)amino]benzoate (16)

Compound **15** (2.0 g, 6.6 mol) was taken in ethanol (50 mL) and hydrogenated at 30 psi in the presence of platinum oxide (0.1 g) for 4 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated to dryness. The residue was recrystallized from methanol–ethyl acetate–hexane to give 0.8 g (44%) of **16** as a light brown powder, mp 150–155 °C. ^{1}H NMR (DMSO-d_6): δ 8.63 (s, 1H), 7.37 (d, J = 2.0 Hz, 1H), 7.30 (d, J = 2.0 Hz, 1H), 5.37 (br s, 2H), 5.10 (br s, 1H), 4.60 (s, 2H), 3.81 (s, 3H), 3.04 (s, 3H). IR (KBr): 3472, 3269, 1704, 1436, 1307 cm^{-1}. MS (ES$^+$), *m/z*: 257.1. Anal. ($C_{10}H_{14}N_2O_5S$) C, H, N.

4.10. 3-(Aminoiminomethyl)amino-5-hydroxymethyl-4-(methylsulfonyl)aminobenzoic acid hydrate (17)

A mixture of compound **16** (0.548 g, 2.0 mmol), 4 N hydrochloric acid in dioxane (0.6 mL, 2.4 mmol) and cyanamide (0.84 g, 20.0 mmol) in ethyl acetate (20 mL) was heated at reflux for 16 h. A brown-colored cake was deposited at the bottom of the flask. The supernatant was removed by decantation and the cake was stirred with fresh ethyl acetate (20 mL) at room temperature for 4 h. The brown solid was collected by filtration and dried to give 0.52 g (73%) of methyl 3-(aminoiminomethyl)amino-5-hydroxymethyl-4-(methylsulfonyl)aminobenzoate hydrochloride. This ester (0.5 g, 1.4 mmol) was stirred with 1 N sodium hydroxide (3.0 mL, 3.0 mmol) at room temperature for 16 h and the mixture filtered through a cotton plug. The filtrate was neutralized with concd hydrochloric acid. A fine yellow precipitate was obtained, which was removed by filtration and the filtrate was evaporated to dryness. The residue was taken in methanol, insolubles removed by filtration and the filtrate evaporated to dryness. The residue was again dissolved in methanol and isopropanol was added to it. The precipitate, which formed was collected by filtration to give 0.06 g (13%) of **17** as a fluffy powder, mp 300–307 °C. ^{1}H NMR (DMSO-d_6): δ 8.15 (br s, 4H), 7.93 (s, 1H), 7.68 (s, 1H), 4.62 (s, 2H), 2.81 (s, 3H). IR (KBr): 3348, 1617, 1559, 1386, 1315 cm^{-1}. MS (ES$^+$), *m/z*: 303.2. Anal. ($C_{10}H_{14}N_4O_5S{\cdot}H_2O{\cdot}0.1[(CH_3)_2CH]_2O$) C, H, N.

4.11. Methyl 3-[(N-hydroxylimino)methyl]-4-[(methylsulfonyl)amino]-5-nitrobenzoate (18)

A mixture of compound **15** (0.151 g, 0.5 mmol) and hydroxylamine hydrochloride (0.07 g, 1.0 mmol) in ethanol (5 mL) was heated at reflux for 2 h. The mixture was filtered hot and the filtrate was concentrated to dryness. The residue was suspended in water. The solid was collected by filtration and recrystallized from methanol to give 0.11 g (68%) of **18** as light yellow needles, mp 208 °C. ^{1}H NMR (DMSO-d_6): δ 12.01 (s, 1H), 10.26 (s, 1H), 8.59 (d, J = 2.0 Hz, 1H), 8.45 (s, 1H), 8.37 (d, J = 2.0 Hz, 1H), 3.92 (s, 3H), 3.07 (s, 3H). IR (KBr): 3423, 3270, 1721, 1347, 1160 cm^{-1}. MS (ES$^+$), *m/z*: 318.4. Anal. ($C_{10}H_{11}N_3O_7S$) C, H, N.

4.12. Methyl 3-amino-5-[(N-hydroxyimino)methyl]-4-[(methylsulfonyl)amino]benzoate (19)

Compound **18** (1.0 g, 3.15 mmol) was dissolved in ethanol (30 mL) and hydrogenated at 40 psi in the presence of platinum oxide (50 mg) for 2 h. The white, crystalline material separated out along with the catalyst, which was collected by filtration and dissolved in methanol with heating. The mixture was filtered through Celite to remove the catalyst and the filtrate gave on cooling 0.4 g (44%) of **19** as pale yellow crystals, mp 218 °C. ^{1}H NMR (DMSO-d_6): δ 11.28 (s, 1H), 8.92 (s, 1H), 8.34 (s, 1H), 7.63 (d, J = 2.0 Hz, 1H), 7.39 (d, J = 2.0 Hz, 1H), 5.58 (s, 2H), 3.82 (s, 3H), 3.02 (s, 3H). IR (KBr): 3502, 3328, 1707, 1631, 1320, 1235 cm^{-1}. MS (ES$^+$), *m/z*: 288.0. Anal. ($C_{10}H_{13}N_3O_5S$) C, H, N.

4.13. Methyl 5-[(*t*-butoxycarbonylamino-*t*-butoxycarbonylimino)methyl]amino-3-[N-(hydroxy)imino]methyl-4-N-(methylsulfonyl)amino benzoate (20)

To a solution of **19** (0.813 g, 2.83 mmol) in dimethylformamide (3 mL) was added triethylamine (1.4 mL, 9.9 mmol) and bisBocthiourea (0.83 g, 2.97 mmol). The resulting mixture was cooled to 0 °C and mercuric chloride (0.86 g, 3.12 mmol) was added. The reaction mixture was stirred at 0 °C for 30 min and then at room temperature for 2 h. Ethyl acetate (25 mL) was added and the slurry filtered through Celite. The filtrate was washed with water (2 × 20 mL) and brine (10 mL), the organic layer was dried (Na_2SO_4), filtered and solvent removed in vacuo to give 1.68 g of crude product. The crude product was purified by flash column chromatography (40% ether in hexane) to obtain 0.3 g (20%) of **20**, mp 143–145 °C. ^{1}H NMR (DMSO-d_6): δ 11.64 (s, 1H), 11.59 (s, 1H), 10.51 (s, 1H), 9.57 (s, 1H), 8.62 (s, 1H), 8.42 (s, 1H), 8.24 (s, 1H), 3.87 (s, 3H), 3.12 (s, 3H), 1.52 (s, 9H), 1.39 (s, 9H). IR (KBr): 3230, 2981, 1729, 1654, 1614, 1336, 1154 cm^{-1}. MS (ES$^-$), *m/z*: 528.4. Anal. ($C_{21}H_{31}N_5O_9S$) C, H, N.

4.14. 5-[(Aminoimino)methyl]amino]-3-[N-(hydroxyl)imino]methyl-4-(methylsulfonyl)-amino benzoic acid (21)

To a solution of **20** (0.24 g, 0.46 mmol) in methylene chloride (5 mL) was added dropwise trifluoroacetic acid (0.9 mL, 11.5 mmol) and stirred at room temperature for 16 h. After 16 h, additional trifluoroacetic acid (0.9 mL, 11.5 mmol) was added and the reaction stirred for 1 h at room temperature. The solvent was removed in vacuo and the excess trifluoroacetic acid was removed

by co-distilling thrice under vacuo with methylene chloride (10 mL). The resulting residue was dissolved in 1 M NaOH (2.3 mL, 2.3 mmol) and stirred at room temperature for 1 h. The reaction mixture was filtered and concentrated to 1 mL. The pH was adjusted to 6 using glacial acetic acid. The solid separated on standing was collected by filtration and washed with water, and dried to furnish 0.1 g (72%) of **21** as a white solid, mp 254 °C. ^{1}H NMR (DMSO-d_6): δ 8.47 (s, 1H), 8.22 (d, J = 1.7 Hz, 1H), 7.70–7.90 (br s, 4H), 7.74 (d, J = 1.6 Hz, 1H), 2.80 (s, 3H). IR (KBr): 3405, 3159, 1698, 1656, 1549, 1379 cm^{-1}. MS (ES$^+$), *m/z*: 316.2. Anal. ($C_{10}H_{13}N_5O_5S$) C, H, N.

4.15. 2-(Ethoxycarbonyl)-5-(methanesulfonylamino)pyridine (23)

Compound **22** (0.8 g, 4.8 mmol) was dissolved in dichloromethane (12.0 mL) containing pyridine (0.432 mL, 5.3 mmol) and the solution cooled to 0 °C. Methanesulfonyl chloride (0.408 mL, 5.3 mmol) was added to the solution over a 10 min period. This temperature was maintained over a 30 min period after which the reaction was stirred at room temperature for 16 h. The reaction contents were poured into water, the organic layer was separated and then washed twice with water (40 mL). The dichloromethane layer was then concentrated. The resulting solid was suspended in water and then collected by filtration. The solid was recrystallized from ethyl acetate. A second batch of crystals was obtained by adding hexane to the ethyl acetate filtrate. The combined, filtered batches of product were dried in vacuo to give 0.763 g (65%) of **23**, as an orange solid, mp 168–169 °C. ^{1}H NMR (DMSO-d_6): δ 10.60 (s, 1H), 8.55 (d, J = 2.0 Hz, 1H), 8.15 (d, J = 6.7 Hz, 1H), 7.75 (dd, J = 6.7 Hz, 2.0 Hz, 1H), 4.35 (q, J = 7.1 Hz, 2H), 3.20 (s, 3H), 1.30 (t, J = 7.1 Hz, 3H). IR (KBr): 1713, 1594, 1320, 1231, 1145 cm^{-1}. MS (ES$^+$), *m/z*: 245.4. Anal. ($C_9H_{12}N_2O_4S$) C, H, N.

4.16. 5-(Methylsulfonylamino)pyridine-2-carboxylic acid (24)

Compound **23** (0.55 g, 2.3 mmol) was dissolved in 1 N NaOH (2.7 mL, 2.7 mmol) and the reaction stirred for 20 h at room temperature. Additional 1 N NaOH (1.13 mL, 1.1 mmol) was added to the reaction. The solution was acidified with concentrated HCl and then filtered to give 0.349 g (72%) of **24**. An analytical sample was prepared by re-crystallization from *n*-butanol and the solid was washed with ether to give pure **24** as a pale yellow solid, mp 235–237 °C. ^{1}H NMR (DMSO-d_6): δ 10.6 (s, 1H), 8.50 (d, J = 2.0 Hz, 1H), 8.05 (d, J = 6.8 Hz, 1H), 7.80 (dd, J = 6.8 Hz, 2.0 Hz, 1H), 3.15 (s, 3H). IR (KBr): 3130, 1685, 1592, 1390, 1153 cm^{-1}. MS (ES$^-$), *m/z*: 215.0. Anal. ($C_7H_8N_2O_4S$) C, H, N.

4.17. Ethyl 5-acetylaminopyridine-2-carboxylate (25)

A mixture of **22** (1.5 g, 9.0 mmol), acetic anhydride (5.0 mL) and glacial acetic acid (10.0 mL) was heated at reflux for 3 h. The mixture was concentrated on rotavap, the residual acetic acid was removed by repeated co-evaporation with water and the residue recrystallized from ethyl acetate to give 1.5 g (80%) of **25** as a light brown solid, mp 147–148 °C. ^{1}H NMR (DMSO-d_6): δ 10.50 (s, 1H), 8.78 (d, J = 1.9 Hz, 1H), 8.25 (dd, J = 10.2, 2.4 Hz, 1H), 8.02 (d, J = 10.5 Hz, 1H), 4.31 (q, J = 7.0 Hz, 2H), 2.11 (s, 3H), 1.31 (t, J = 7.0 Hz, 3H). IR (KBr): 1727, 1697, 1540, 1381, 1294 cm^{-1}. MS (ES$^+$), *m/z*: 209.5. Anal. ($C_{10}H_{12}N_2O_3$) C, H, N.

4.18. 5-Acetylaminopyridine-2-carboxylic acid, sodium salt (26)

A mixture of **25** (1.04 g, 5.0 mmol) in 1 N sodium hydroxide (7 mL, 7.0 mmol) was stirred at room temperature for 4 h. The mixture was neutralized with H+ resin, filtered and the filtrate concentrated to dryness. The residue was recrystallized from water–ethanol to give 0.6 g (62%) of **26** as an off-white powder, mp 300 °C. ^{1}H NMR (D_2O): δ 8.84 (d, J = 1.8 Hz, 1H), 8.19 (dd, J = 6.4, 2.2 Hz, 1H), 8.08 (d, J = 6.5 Hz, 1H), 2.22 (s, 3H). IR (KBr): 3244, 1709, 1680, 1604, 1552, 1388 cm^{-1}. MS (ES$^+$), *m/z*: 181.1. Anal. ($C_8H_8N_2O_3 \cdot 0.5Na$) C, H, N.

4.19. Ethyl 5-thioxoethylaminopyridine-2-carboxylate (27)

A mixture of **25** (0.416 g, 2.0 mmol) and Lawesson's reagent (0.405 g, 1.0 mmol) in toluene (20 mL) was heated at reflux for 4 h. Toluene was evaporated and the residue passed through a column of silica gel using ethyl acetate as eluent. The appropriate fractions were mixed together, concentrated and the residue recrystallized from ethyl acetate to give 0.34 g (76%) of **27** as a yellow powder, mp 159–160 °C. ^{1}H NMR (DMSO-d_6): δ 11.99 (s, 1H), 9.00 (d, J = 2.3 Hz, 1H), 8.67 (dd, J = 8.0, 2.3 Hz, 1H), 8.12 (d, J = 8.0 Hz, 1H), 4.34 (q, J = 7.1 Hz, 2H), 2.66 (s, 3H), 1.33 (t, J = 7.1 Hz, 3H). IR (KBr): 3290, 3113, 1722, 1355, 1303, 1164 cm^{-1}. MS (ES$^+$), *m/z*: 225.4. Anal. ($C_{10}H_{12}N_2O_2S$) C, H, N.

4.20. 5-Thioxoethylaminopyridine-2-carboxylic acid, hydrate (28)

A mixture of **27** (112 mg, 0.5 mmol) was stirred with 1 N sodium hydroxide (1.2 mL, 1.2 mmol) at room temperature for 4 h and the mixture filtered through a cotton plug. The filtrate was neutralized with dilute hydrochloric acid. Yellow precipitate separated out which was collected by filtration, washed with water and dried to give 75 mg (75%) of **28** as a yellow powder, mp 184 °C (dec., starts darkening at 100 °C). ^{1}H NMR (DMSO-d_6): δ 11.99 (s, 1H), 9.00 (d, J = 2.3 Hz, 1H), 8.62 (dd, J = 8.0, 2.3 Hz, 1H), 8.09 (d, J = 8.0 Hz, 1H), 2.67 (s, 3H). IR (KBr): 3100, 1720, 1550, 1345 cm^{-1}. MS (ES$^+$), *m/z*: 197.2. Anal. ($C_8H_8N_2O_2S \cdot 0.25H_2O$) C, H, N.

4.21. 2-Amino-6-[(β-N,N-dimethylamino)vinyl]-3-nitropyridine (30)

A mixture of **29** (3.06 g, 20.0 mmol) and dimethylformamide dimethylacetal (6.4 g, 94%, 50.0 mmol) in dimethylformamide (30 mL) was heated at 140 °C for 16 h. The mixture was concentrated under vacuum at 70–80 °C.

The residue was poured into water (60 mL), stirred for 16 h and the red colored precipitate was collected by filtration. It was recrystallized from methanol to give 1.6 g (38.5%) of **30** as maroon needles, mp 183–185 °C. ^{1}H NMR (DMSO-d_6): δ 7.95 (d, J = 9.0 Hz, 1H), 7.80 (d, J = 12.8 Hz, 1H), 6.41 (d, J = 9.0 Hz, 1H), 5.14 (d, J = 12.8 Hz, 1H), 2.98 (br s, 6H). IR (KBr): 1587, 1367, 1251 cm^{-1}. MS (ES$^+$), *m/z*: 209.4. Anal. ($C_9H_{12}N_4O_2$) C, H, N.

4.22. Methyl 6-amino-5-nitropyridine-2-carboxylate (31)

To a mixture of **30** (1.04 g, 5.0 mmol) and potassium carbonate (1.38 g, 10.0 mmol) in aqueous *tert*-butanol (1:1, 10.0 mL) was added powdered potassium permanganate (1.58 g, 10.0 mmol) over a period of 10 min. The mixture was further stirred for 3 h. The solids were separated by filtration and the filtrate was concentrated to dryness to give 1.8 g of crude 6-amino-5-nitropyridine-2-carboxylic acid.

The crude 6-amino-5-nitropyridine-2-carboxylic acid (1.8 g) was suspended in methanol (50.0 mL) and concentrate sulfuric acid (2.0 mL) was added to it carefully. The mixture was heated at reflux for 4 h. On cooling, the mixture was poured into ice water. The yellow precipitate formed, which was collected by filtration and recrystallized from ethyl acetate–hexane to give 0.40 g (41%) of **31** as a yellow solid, mp 192 °C. ^{1}H NMR (DMSO-d_6): δ 8.54 (d, J = 8.5 Hz, 1H), 8.09 (br s, 2H), 7.29 (d, J = 8.5 Hz, 1H), 3.88 (s, 3H). IR (KBr): 3483, 3276, 1733, 1634, 1239 cm^{-1}. MS (ES$^+$), *m/z*: 198.4. Anal. ($C_7H_7N_3O_4$) C, H, N.

4.23. 2,3-Diamino-6-methoxycarbonylpyridine (32)

A mixture of **31** (1.3 g, 6.6 mmol) in ethanol (30 mL) was hydrogenated in the presence of platinum oxide (0.1 g) at 30 psi for 0.5 h. The catalyst was removed by filtration through Celite and the filtrate was concentrated. The residue was recrystallized from ethyl acetate to give 1.0 g (96%) of **32** as a brown solid, mp 113–116 °C. ^{1}H NMR (DMSO-d_6): δ 7.22 (d, J = 7.8 Hz, 1H), 6.68 (d, J = 7.8 Hz, 1H), 5.74 (br s, 2H), 5.52 (br s, 2H), 3.71 (s, 3H). IR (KBr): 3415, 3165, 1720, 1635, 1476, 1252 cm^{-1}. MS (ES$^+$), *m/z*: 167.9. Anal. ($C_7H_9N_3O_2$) C, H, N.

4.24. 3-Acetylamino-2-amino-6-methoxycarbonylpyridine (33)

To a stirred mixture of **32** (570 mg, 3.4 mmol) in tetrahydrofuran (20 mL) and pyridine (558 mg, 7.0 mmol), acetic anhydride (381 mg, 3.74 mmol) was added at 0 °C over a period of 10 min. The mixture was stirred for 0.5 h at 0 °C and then at room temperature for 16 h. The mixture was concentrated and the residue was passed through a column of silica gel using ethyl acetate: methanol (9:1) as eluent. The appropriate fractions were combined and concentrated. The residue was recrystallized from ethyl acetate to give 350 mg (49%) of **33** as brown crystals, mp 184–184 °C. ^{1}H NMR (DMSO-d_6): δ 9.21 (s, 1H), 7.97 (d, J = 8.0 Hz, 1H), 7.30 (d, J = 8.0 Hz, 1H), 6.25 (s, 2H), 3.79 (s, 3H), 2.09 (s, 3H). IR (KBr): 3457, 3251, 1744, 1654, 1534, 1469 cm^{-1}. MS (ES$^+$), *m/z*: 209.9. Anal. ($C_9H_{11}N_3O_3$) C, H, N.

4.25. 5-Acetylamino-6-aminopyridine-2-carboxylic acid (34)

A mixture of **33** (0.25 g, 1.2 mmol) was stirred with 1 N sodium hydroxide (1.68 mL, 1.68 mmol) at room temperature for 3 h and the mixture filtered through a cotton plug. The filtrate was neutralized with dilute hydrochloric acid. A light brown solid separated out which was collected by filtration, washed with water and dried to give 0.1 g (43%) of **34** as a light brown powder, mp 285–290 °C (dec). ^{1}H NMR (DMSO-d_6): δ 9.25 (s, 1H), 7.94 (d, J = 7.9 Hz, 1H), 7.28 (d, J = 7.9 Hz, 1H), 6.20 (s, 2H), 2.09 (s, 3H). IR (KBr): 3285, 3145, 1679, 1650, 1570, 1389 cm^{-1}. MS (ES$^+$), *m/z*: 196.0. Anal. ($C_8H_9N_3O_3{\cdot}0.75H_2O$) C, H, N.

4.26. 5-Acetylamino-6-[(aminoiminomethyl)amino]pyridine-2-carboxylic acid (35)

A mixture of **33** (0.523 g, 2.5 mmol), concentrate hydrochloric acid (0.42 g, 5.0 mmol) and cyanamide (2.10 g, 5.0 mmol) in ethyl acetate (30 mL) was heated at reflux for 24 h. A brown colored cake was deposited at the bottom of the flask. The supernatant was decanted in another flask and to the supernatant was again added 2 drops of concentrated hydrochloric acid and cyanamide (1.0 g, 2.4 mmol) and heated at reflux for 24 h. The reaction mixture on cooling gave white precipitate, which was collected by filtration and washed with ethyl acetate to give 0.15 g (22%) of methyl 5-acetylamino-6-[(aminoiminomethyl)amino]pyridine-2-carboxylate hydrochloride.

The above ester (0.1 g, 0.35 mmol) was stirred with 1 N sodium hydroxide (1.0 mL, 1.0 mmol) and water (4.0 mL) at room temperature for 4 h and the mixture filtered through a cotton plug. The pH of the filtrate was adjusted close to 8.0 with dilute hydrochloric acid. White solid separated out which was collected by filtration, washed with water and dried to give 0.04 g (46%) of **35** as white powder, mp >300 °C. ^{1}H NMR (ND_4OD): δ 8.69 (d, J = 8.1 Hz, 1H), 7.89 (d, J = 8.1 Hz, 1H), 2.67 (s, 3H). IR (KBr): 3446, 3230, 1735, 1629, 1546, 1378 cm^{-1}. MS (ES$^-$), *m/z*: 236.0. Anal. ($C_9H_{11}N_5$) C, H, N.

4.27. Methyl 4-amino-5-nitropyridine-2-carboxylate (37)

Compound **36** (0.7 g, 3.33 mmol) was dissolved in anhydrous methanol (75 mL) containing concentrated sulfuric acid (1.5 mL) and the mixture heated at reflux for 4 days. The mixture was concentrated to about one-third of its original volume and poured onto chipped ice. The pH was then adjusted to ca. 8 at which time fine yellow needles began to separate. The crystals were collected by filtration and dried to give 0.38 g (58%) of **37**. An analytical sample was prepared by recrystallizing a portion from methanol as yellow needles, mp 223–225 °C. ^{1}H NMR (DMSO-d_6): δ 9.0 (s, 1H), 8.2–8.4 (br s, 2H), 7.65 (s, 1H), 3.86 (s, 3H). IR (KBr): 3485, 3343, 1736,

1627, 1507, 1243 cm^{-1}. MS (ES^-), *m/z*: 196.3. Anal. ($C_7H_7N_3O_4$) C, H, N.

4.28. Methyl 4,5-diaminopyridine-2-carboxylate hydrochloride (38)

Compound **37** (3.15 g, 15.98 mmol) was dissolved in anhydrous methanol (75 mL) containing PtO_2 (0.1 g). The mixture was hydrogenated at 40 psi for 1 h. The mixture was filtered through Celite to remove catalyst and the filtrate concentrated to dryness to give 2.65 g (100%) of **38**. A portion of the compound was dissolved in 1 N HCl (4 equiv) and concentrated to dryness. The residue was recrystallized from water to give **38** as a pale yellow powder, mp 223–225 °C. ^1H NMR (DMSO-d_6): δ 7.84 (br s, 2H), 7.68 (s, 1H), 7.36 (s, 1H), 6.46 (br s, 2H), 3.93 (s, 3H). IR (KBr): 3361, 3176, 1728, 1664, 1579, 1372, 1283 cm^{-1}. MS (ES^+), *m/z*: 168.4. Anal. ($C_7H_9N_3O_2 \cdot HCl$) C, H, N.

4.29. Methyl 5-acetylamino-4-aminopyridine-2-carboxylate hydrate (39)

Compound **38** (2.2 g, 13.17 mmol) was dissolved in a mixture of tetrahydrofuran (80 mL) and dimethylformamide (20 mL). To this solution was added triethylamine (2.2 mL, 15.8 mmol) followed by acetic anhydride (1.36 mL, 14.5 mmol) in one portion. The mixture was stirred for 18 h, concentrated and the residue chromatographed over silica gel using the gradient 5% methanol in ethyl acetate to 10% methanol in ethyl acetate. Concentration of the desired fractions gave 1.5 g (55%) of the amide **39** as an off-white solid. An analytical sample was prepared by recrystallization from acetonitrile–water twice to give **39** as a white powder, mp 167–168 °C. ^1H NMR (DMSO-d_6): δ 9.30 (s, 1H), 8.28 (s, 1H), 7.39 (s, 1H), 6.13 (s, 2H), 3.37 (s, 3H), 2.07 (s, 3H). IR (KBR): 3351, 1668, 1395, 1510 cm^{-1}. MS (ES^+), *m/z*: 210.2. Anal. ($C_9H_{11}N_3O_3 \cdot 0.25H_2O$) C, H, N.

4.30. 5-Acetylamino-4-aminopyridine-2-carboxylic acid hydrate (40)

Compound **39** (0.209 g, 1.0 mmol) was suspended in 1 N NaOH (1.1 mL, 1.1 mmol). After 1 min, the suspension became a clear homogeneous solution. The pH was carefully adjusted to ca. 8 with 1 N HCl and within 0.5 h, fine white needles began to form. The crystals were collected by filtration and a second crop was obtained by adjusting the pH of the filtrate to 7. The first and second crops were combined to give 0.12 g (61.5%) of **40**. An analytical sample was prepared by recrystallization from water as a white powder, mp 284–285 °C. ^1H NMR (DMSO-d_6): δ 9.42 (s, 1H), 8.26 (s, 1H), 7.42 (s, 2H), 7.31 (s, 1H), 2.07 (s, 3H). IR (KBr): 3193, 1698, 1623, 1540, 1369 cm^{-1}. MS (ES^-), *m/z*: 193.9. Anal. ($C_8H_9N_3O_3 \cdot 1.25H_2O$) C, H, N.

4.31. 2,4-Dichloro-6-[β-(N,N-dimethylamino)vinyl]-3-nitropyridine (42)

A sample of 2,4-dichloro-6-methyl-3-nitropyridine, **41** (11.4 g, 55.0 mmol) was dissolved in anhydrous DMF (20 mL) containing *N*,*N*-dimethylformamide dimethylacetal (10.1 mL, 72.0 mmol). The solution was heated to 140 °C for 5 h during which time the mixture became very dark. The mixture was cooled and excess solvents removed in vacuo at 40 °C affording a dark residue. The residue was passed through a short bed of silica gel with hexane (100%, 500 mL) and then ethyl acetate–hexane (1:1, 1500 mL). The ethyl acetate–hexane fractions were combined and concentrated to give a dark red solid, which was crystallized from methanol to give 5.3 g, (37%) of **42** as burnt orange needles, mp 124–125 °C. ^1H NMR ($CDCl_3$): δ 7.68 (d, J = 12.6 Hz, 1H), 6.71 (s, 1H), 4.98 (d, J = 12.6 Hz, 1H), 2.99 (br s, 6H). IR (KBr): 1635, 1532, 1346, 1312 cm^{-1}. MS (ES^-), *m/z*: 233.4, 235.1. Anal. ($C_9H_9Cl_2N_3O_2$) C, H, N.

4.32. 2,4-Dichloro-3-nitropyridine-6-carboxylic acid hydrate (43)

Compound **42** (3.2 g, 12.2 mmol) was dissolved in 50% aqueous *tert*-butyl alcohol (25 mL) containing potassium carbonate (3.7 g, 26.8 mmol). Powdered potassium permanganate (3.85 g, 24.4 mmol) was added portionwise over 0.5 h and the mixture stirred for an additional 5 h. The mixture was diluted with water (25 mL) and excess permanganate destroyed by the addition of sodium bisulfite. The mixture was filtered through Celite and the filtrate concentrated to ca. pH 5 at which time crystals began forming. The crystals were collected by filtration to give 0.884 g and 0.52 g as a second crop (49%) of **43** as yellow needles, mp 137–139 °C. ^1H NMR ($CDCl_3$): δ 8.35 (s, 1H). IR (KBr): 3540, 1721, 1559, 1346 cm^{-1}. MS (ES^-), *m/z*: 234.9, 236.9. Anal. ($C_6H_2Cl_2N_2O_4 \cdot 0.25H_2O$) C, H, N.

4.33. 4,6-Diamino-5-nitropyridine-2-carboxylic acid (44)

Compound **43** (26 g, 0.11 mol) was taken in a pressure bomb with freshly prepared methanolic ammonia (saturated at 5 °C, 500 mL), and the bomb was heated at 100–103 °C for 18 h. The pressure in the bomb reached 160 psi. The bomb was cooled and the yellow precipitate was collected by filtration, washed with methanol and water and dried to give 9.2 g of the product. The combined filtrates were concentrated, suspended in water and neutralized carefully with dilute hydrochloric acid to give yellow precipitate. The precipitate was collected by filtration, washed with water and dried to give 8.6 g of more product. The total yield was 17.8 g (82%) of **44**. An analytical sample was prepared by suspending a small amount in dilute hydrochloric acid, stirring for 1 h, filtering and washing with water to give **44** as a yellow powder, mp 320 °C (dec). ^1H NMR (CF_3CO_2D): δ 7.29 (s, 1H). IR (KBr): 3434, 3109, 1622, 1580, 1286 cm^{-1}. MS (ES^-), *m/z*: 197.0. Anal. ($C_6H_6N_4O_4$) C, H, N.

4.34. Methyl 4,6-diamino-5-nitropyridine-2-carboxylate (45)

A mixture of **44** (5.0 g, 25.2 mmol), methanol (100 mL) and concentrated sulfuric acid (5.0 mL) was heated at reflux for 48 h. The reaction mixture was concentrated

to half of its volume. The crystalline precipitate formed, which was collected by filtration and washed with cold methanol. The cake was suspended in water (100 mL) and triethylamine (10 mL) and the mixture stirred for 1 h. The yellow precipitate was collected by filtration, washed with water and dried to give 5.0 g (93%) of **45**. A small sample was recrystallized from dimethylsulfoxide and water to give **45** as a yellow solid, mp 301 °C (dec). ^{1}H NMR (CF_3CO_2D): δ 7.29 (s, 1H), 4.10 (s, 3H). IR (KBr): 3474, 3349, 3135, 1716, 1614, 1550 cm^{-1}. MS (ES^-), *m/z*: 211.0. Anal. ($C_7H_8N_4O_4$) C, H, N.

4.35. Methyl 4,5,6-triaminopyridine-2-carboxylate hydrochloride (46)

A mixture of **45** (4.8 g, 22.6 mmol) in methanol (200 mL) and chloroform (10 mL) was hydrogenated at 38 psi in the presence of platinum oxide (0.6 g) for 8 h. The mixture was filtered through Celite and the filtrate was concentrated to half of its volume. The crystalline precipitate formed, which was collected by filtration and recrystallized from methanol to give 1.5 g (30%) of **46** as a pale yellow solid, mp 292–294 °C (dec). Another crop of 0.8 g (16%) was also collected from the filtrate. ^{1}H NMR (DMSO-d_6): δ 7.21 (s, 2H), 7.05 (s, 1H), 6.77 (s, 2H), 5.59 (s, 2H), 3.88 (s, 3H). IR (KBr): 3387, 3219, 3190, 1735, 1653 cm^{-1}. MS (ES^+), *m/z*: 182.9. Anal. ($C_7H_{10}N_4O_2$·HCl) C, H, N.

4.36. Methyl 4,6-diamino-5-methylsulfonylaminopyridine-2-carboxylate (47)

Compound **46** (3.73 g, 17.0 mmol) was suspended in pyridine (100 mL) and methanesulfonyl chloride (2.58 g, 22.0 mmol) was added with syringe over a period of 10 min. The reaction mixture was stirred at room temperature for 6 h. The reaction mixture was concentrated, the residue was purified twice through a column of silica gel using [dichloromethane (70–50%)] in a mixture of [chloroform (80)–methanol (18)–ammonium hydroxide (2)]. The appropriate fractions were pooled together and concentrated. The residue was recrystallized from methanol to give 0.15 g (2.6%) of **47** as a white powder, mp 232–234 °C (dec). ^{1}H NMR (DMSO-d_6): δ 8.39 (s, 1H), 6.80 (s, 1H), 5.93 (br s, 2H), 5.76 (br s, 2H), 3.76 (s, 3H), 3.03 (s, 3H). IR (KBr): 3448, 3363, 1711, 1627, 1596, 1451 cm^{-1}. MS (ES^+), *m/z*: 261.2. Anal. ($C_8H_{12}N_4O_4S$) C, H, N.

4.37. 4,6-Diamino-5-methylsulfonylaminopyridine-2-carboxylic acid (48)

Compound **47** (70 mg, 0.27 mmol) was stirred with 0.5 N sodium hydroxide (1.0 mL, 0.5 mmol) for 4 h. The mixture was filtered through a plug of cotton and the filtrate was neutralized with acetic acid. The precipitate formed, which was collected by filtration, washed with water and dried to give 45 mg (68%) of **48** as an off-white solid, mp >330 °C. ^{1}H NMR (CF_3CO_2D): δ 7.33 (s, 1H), 3.38 (s, 3H). IR (KBr): 3472, 3354, 1621, 1465, 1370 cm^{-1}. MS (ES^+), *m/z*: 247.0. Anal. ($C_7H_{10}N_4O_4S$) C, H, N.

4.38. Methyl 5-acetylamino-4,6-diaminopyridine-2-carboxylate hydrochloride (49)

Compound **46** (2.0 g, 9.1 mmol) was dissolved in pyridine (30 mL) by heating. The mixture was cooled in an ice bath and acetyl chloride (0.9 mL, 12.7 mmol) was added to it with syringe over a period of 5 min. The reaction mixture was stirred at 0–5 °C for 0.5 h and then at room temperature for 1 h. Pyridine was evaporated under vacuum, the residue was taken in methanol (20 mL), ethyl acetate (20 mL) added and the mixture left overnight in the refrigerator. The precipitate was collected by filtration, washed with ethyl acetate and recrystallized from methanol to give 1.0 g (42%) of **49** as an off-white powder, mp 275–280 °C (dec). ^{1}H NMR (DMSO-d_6): δ 9.05 (s, 1H), 7.31 (br s, 4H), 6.98 (s, 1H), 3.92 (s, 3H), 2.05 (s, 3H). IR (KBr): 3129, 1728, 1655, 1605, 1285 cm^{-1}. MS (ES^+), *m/z*: 225.1. Anal. ($C_9H_{12}N_4O_3$·HCl) C, H, N.

4.39. Methyl 5-acetylamino-4-amino-6-[(*t*-butoxycarbonylamino-*t*-butoxycarbonyl-imino)-methyl]aminopyridine-2-carboxylate (50)

A mixture of **49** (0.91 g, 3.5 mmol), N,N′-bis(*t*-butoxycarbonyl)thiourea (0.966 g, 3.5 mmol) and triethylamine (1.24 g, 12.3 mmol) in dimethylformamide (10.0 mL) was cooled to 0 °C. Mercuric chloride (0.95 g, 3.5 mmol) was added to the mixture and stirred at 0 °C for 0.5 h and then at room temperature for 16 h. The mixture was diluted with ethyl acetate (100 mL) and filtered through Celite. The filtrate was washed with brine, organic layer dried over sodium sulfate and concentrated. The residue was passed through a column of silica gel using ethyl acetate–hexane as eluent. The appropriate fractions were collected, concentrated and dried to give 0.22 g (13.5%) of **50** as a white powder, mp >320 °C (compound darkens above 250 °C. ^{1}H NMR (DMSO-d_6): δ 12.60 (s, 1H), 11.83 (s, 1H), 9.20 (s, 1H), 7.22 (s, 1H), 6.66 (s, 2H), 3.84 (s, 3H), 2.10 (s, 3H), 1.48 (s, 9H), 1.44 (s, 9H). IR (KBr): 3445, 3298, 1760, 1724, 1676 cm^{-1}. MS (ES^+), *m/z*: 467.9. Anal. ($C_{20}H_{30}N_6O_7$) C. H, N.

4.40. 5-Acetylamino-4-amino-6-[(aminoiminomethyl)-amino]pyridine-2-carboxylic acid (51)

Compound **50** (180 mg, 0.38 mmol) was dissolved in dry dichloromethane (15 mL) and trifluoroacetic acid (1.0 mL) was added to it. The mixture was concentrated after it was stirred at room temperature for 16 h. The residual amounts of trifluoroacetic acid were removed by several co-evaporations with dichloromethane to give a white powder (180 mg) of methyl 5-acetylamino-4-amino-6-(aminoiminomethyl)pyridine-2-carboxylate trifluoroacetate, which was suspended in 1 N sodium hydroxide (1.0 mL) and water (1.2 mL) and the mixture stirred for 2 h. It was filtered through a plug of cotton and pH was brought close to 9.0 by careful addition of dilute hydrochloric acid. White precipitate obtained was collected by filtration, washed with water and dried to give 50 mg (51%) of **51** as a white powder, mp >320 °C (compound darkens above 250 °C). ^{1}H NMR

(CF_3CO_2D): δ 7.79 (s, 1H), 2.37 (s, 3H). IR (KBr): 3420, 3309, 1699, 1629, 1580 cm^{-1}. Anal. ($C_9H_{12}N_6O_3 \cdot 0.25H_2O$) C, H, N.

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