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Effect of substituent structure on pyrimidine electrophilic substitution: a rebuttal

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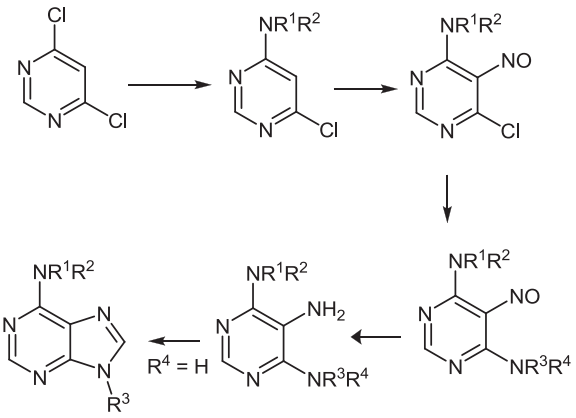
ABSTRACT

The report about a series of unexpected and obscure effects influencing the electrophilic nitrosation of activated pyrimidines (*Tetrahedron* **2007**, *63*, 5394) was shown to be erroneous. Instead of electrophilic substitution at position 5 of the pyrimidine ring, N-nitrosation of the secondary amino group in the 4-position of the pyrimidine ring took place. Moreover it was shown that the synthetic sequence for the preparation of purines is also incorrect.

1. Introduction

For a long time, 5-nitrosopyrimidines remain important intermediates in the synthesis of diverse pyrimidine containing heterocycles.[1] It is also known that 5-nitrosopyrimidines have unique crystal structures[2] and they can act as bidentate ligands for coordination of metal ions.[3] Various alkoxy- and amino-substituted 5-nitrosopyrimidines are important as potential inhibitors of the human DNA-repair protein O^6-alkylguanine-DNA-transferase.[4] Moreover it was found that 5-nitrosopyrimidine derivative NU6027 acts as both a CDK1 and a CDK2 inhibitor with K_i values of 2.5 and 1.3 μM, respectively.[5] In addition, NU6027 showed growth inhibitory activity against human tumor cells with mean GI_{50} value of 10 μM.[6] Recently, we discovered a series of N^4,N^6-disubstituted-5-nitrosopyrimidine-4,6-diamines and some of them caused significant growth inhibition in solid human cancer cell lines with GI_{50} values in the range 3.1–7.2 μM.[7]

It is well known, that synthetically and biologically important 5-nitrosopyrimidines can be prepared by the direct nitrosation reaction of pyrimidines, bearing at least three electron-donating groups in the 2, 4 and 6-positions.[5a,6,8] On the other hand, N^4,N^6-disubstituted-5-nitrosopyrimidine-4,6-diamines can be prepared via intramolecular oxidation-reduction reaction of *N*-(5-nitropyrimidin-4-yl)glycinates discovered by us recently.[9] In 2007 a paper entitled 'Effect of substituent structure on pyrimidine electrophilic substitution' by van der Westhuyzen et al. was published.[10] The paper dealt with direct nitrosation of several *N*-substituted-6-chloropyrimidin-4-amines with explanation of a series of unexpected and obscure effects influencing the electrophilic substitution reactions. The results reported by the authors in this paper were interesting as well as unusual to us. According to the procedures reported in this paper, various 5-nitrosopyrimidines were prepared and used in the synthesis of purines by the sequence presented in Scheme 1. As continuation of our ongoing program aimed at the synthesis of 5-nitrosopyrimidines with notable antitumor activity,[7] we tried to apply van der Westhuyzen's method in our work and found that direct nitrosation at position 5 of the pyrimidine ring is impossible. Herein we report the results of our investigations.



Scheme 1. Synthesis of *N*,6-disubstituted-5-nitrosopyrimidin-4-amines and their utility for preparation of purines, proposed by van der Westhuyzen et al.[10]

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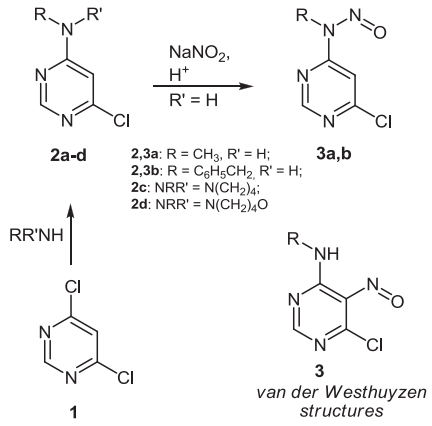
2. Results and discussions

First of all, we prepared the starting materials **2a**–**d** by the reaction of commercially available 4,6-dichloropyrimidine (**1**) with an excess of methylamine (for compd **2a**), benzylamine (for compd **2b**), pyrrolidine (for comps **2c**) or morpholine (for compd **2d**) in boiling 2-propanol. After the nitrosation reaction of **2a**,**b** under the van der Westhuyzen conditions we obtained yellow solids **3a** and **3b** in good yields, as it was stated in their manuscript.[10] However, the color of the obtained products was not common for 5-nitrosopyrimidines (normally, solutions of 5-nitrosopyrimidines are of deep green or deep blue colors). In the IR spectra of compounds **3a**,**b** there were no NH absorption bands. The ^{1}H NMR spectra showed the presence of two sharp singlets (non-exchangeable with D_2O) at 8.06–8.11 and 8.86–8.94 ppm, respectively. Moreover, protons of methyl and methylene groups were uncoupled to NH protons, and appeared as singlets at 3.46 and 5.38 ppm, respectively. In the ^{13}C NMR spectra of compounds **3a**,**b** there were signals at 107.6–107.9 ppm, which cannot be assigned to the C-5 of 5-nitrosopyrimidines, as stated by van der Westhuyzen et al.[10] (normally, C-(5) in 5-nitrosopyrimidines appear at lower fields, approx. at 140 ppm[5a,6,8]). And finally, in the HSQC spectra of **3a**,**b** cross-peaks between signals at 8.06–8.11 ppm and 107.6–107.9 ppm appeared, which is not consistent with the formation of 6-chloro-5-nitrosopyrimidines. These data together with HRMS data indicated that under the reaction conditions the N-nitrosation reaction took place. Inevitably, compounds **2c**,**d** without free NH functionality were unreactive toward nitrosation conditions (Scheme 2).



Scheme 2. Nitrosation reaction of *N*-substituted-6-chloropyrimidin-4-amines.

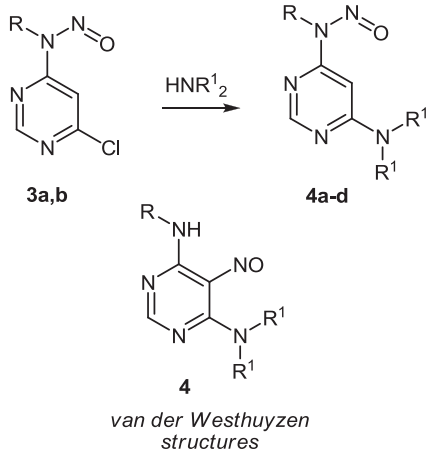
The spectroscopic data of our synthesized compounds **3a**,**b** and compounds, synthesized by van der Westhuyzen et al. were the same, so it is evident, that the authors wrongly elucidated structures of products **3**.

It is well known that electrophilic aromatic substitution at C-5 of the pyrimidine ring is often problematic. The reason for this behavior was identified in the presence of two nitrogen atoms in the structure.[11] An electrophilic reagent, or a proton in the reaction medium, added preferentially to the pyridine nitrogen, generating a pyridinium cation, which is naturally very resistant to a further attack by an electrophile.[12] Therefore, pyrimidines normally require the presence of activating groups or harsh reactions conditions for successful introduction of an electrophile.[13] Nitrous acid cannot be used under vigorous conditions, so that pyrimidine substrates for nitrosation need at least two, but preferably three electron-donating groups to assist the process.[5a,6,8] Therefore, the N-nitrosation reaction of compounds **2a**,**b** took place readily and on the other hand, the starting pyrimidines **2c**,**d** were completely unreactive toward nitrosation conditions.

Moreover, we tried to introduce a 5-nitrogen moiety into the starting compounds **2a**,**b** by direct nitration or coupling with 4-chlorophenyldiazonium salt.[13,14] Diazotization of **2a**,**b** did not give any satisfactory result: after the work-up of the reaction mixtures the initial products were recovered together with small amounts of *N*-nitrosated products **3a**,**b** and 4-chlorophenol, which formed after decomposition of 4-chlorophenyldiazonium salt in water. 6-chloro-*N*-methylpyrimidin-4-amine **2a** was unreactive toward classical nitration conditions (mixture of concd H_2SO_4 and HNO_3), on the other hand *N*-benzyl-6-chloropyrimidin-4-amine **2b** underwent smooth nitration on the phenyl ring (mainly at fourth position). These results showed that there is insufficient electron donation in the starting compounds **2**, so the heterocyclic ring is not activated enough for electrophilic substitution.

Interestingly, the utility of the obtained nitrosation products **3** in the synthesis of purine derivatives was shown in the same manuscript.[10] So a careful check of the procedures reported by van der Westhuyzen et al. was carried out in our laboratory.

N-substituted-6-chloro-*N*-nitrosopyrimidin-4-amines **3a**,**b** were treated with secondary amines pyrrolidine and morpholine in dichloromethane or in dimethylformamide at room temperature, smoothly generating yellowish products **4a**–**d** (Scheme 3, Table 1). The structures of all compounds **4a**–**d** were substantiated by their spectroscopic data. In the ^{1}H NMR spectra of **4a**–**d** a singlet at 8.49–8.61 ppm due to the resonance of C(2)–H along with a singlet at 6.85–7.18 ppm for the C(5)–H and signals of substituents in position 6 of the pyrimidine ring were observed. Signals of CH_3N and PhCH_2N moieties appeared as singlets at 3.43–3.44 ppm and 5.40–5.41 ppm, respectively. The absence of NH absorption bands in the IR spectra of **4a**–**d** and the cross-peak between signals at 5.41 ppm and 87.71 ppm in the HSQC spectra of **4d** together with HRMS data confirmed the structures of nucleophilic displacement reaction products **4**. It should be noted that the spectroscopic data of our synthesized compounds are in agreement with the published herein,[10] so we obtained exactly the same compounds as van der Westhuyzen group.



Scheme 3. Nucleophilic displacement reaction of *N*-substituted-6-chloro-*N*-nitroso pyrimidin-4-amines.

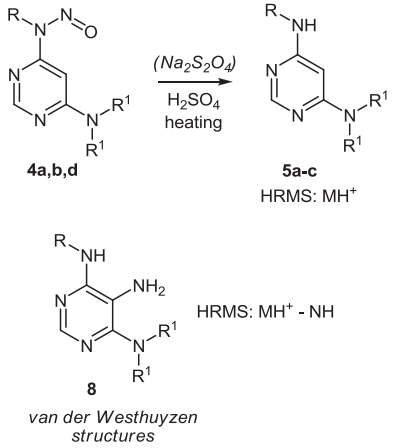
In the light of our obtained results we became particularly intrigued by the Section 2.3 of the van der Westhuyzen's et al. manuscript[10] dealing with reduction of 5-nitrosopyrimidines and subsequent ring closure to the corresponding purines. Therefore we performed the reduction of compounds **4a**,**b**,**d** by van der Westhuyzen's conditions. Treating warm mixtures of compounds **4a**,**b**,**d** in aqueous sulfuric acid with sodium dithionite afforded

Table 1
N, 6-Disubstituted-*N*-nitrosopyrimidin-4-amines **4a**–**d** produced via Scheme 3

Starting comp.	Amine	Product	Product number in *Tetrahedron* **2007**, 5394.	Yield %
3a	$HN(CH_2)_4$	**4a**	**4b**	97
3b	$HN(CH_2)_4$	**4b**	**4d**	92
3a	$HN(CH_2)_4O$	**4c**	n/s	91
3b	$HN(CH_2)_4O$	**4d**	**4e**	96

n/s—not synthesized.

readily filterable solids **5a**–**c**. It should be noted, that the same products **5a**–**c** were formed during heating mixtures of starting compounds **4a**,**b**,**d** in 10% sulfuric acid without addition of sodium dithionite. The spectroscopic data of our synthesized compounds and careful study of van der Westhuyzen's spectroscopic data showed that under these reaction conditions smooth and high-yielding N-denitrosation reactions took place (Scheme 4, Table 2). The IR spectra of **5a**–**c** exhibited absorption at 3209–3243 cm^{-1}, which can be attributed to the absorption of the NH bond. In the ^1H NMR spectra of **5a**–**c** a singlet at 8.11–8.12 ppm due to the resonance of C(2)–H along with a singlet at 5.13–5.38 ppm for the C(5)–H were observed. Signals of CH_3NH and $PhCH_2NH$ moieties appeared as doublets at 2.85 ppm and 4.42–4.43 ppm with coupling constants 5.4–5.7 Hz, respectively. The van der Westhuyzen's and coauthors discussions about problems with compounds stability under the electrospray mass spectroscopic conditions do not have any significance, because found masses are in good agreement with masses of denitrosation products **5**, and there is no need to theorize about smooth fragmentation of molecular ion to MH^+–NH (Scheme 4).

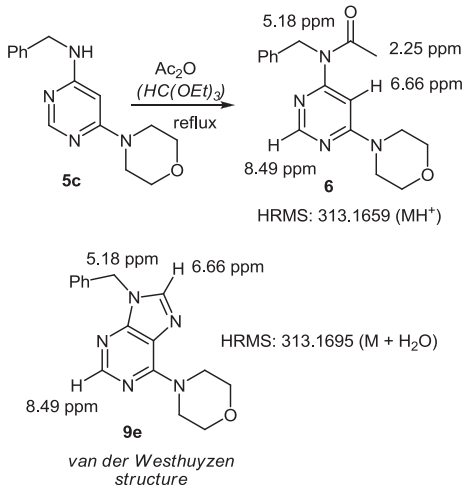


Scheme 4. Denitrosation reaction of *N*,6-disubstituted-*N*-nitrosopyrimidin-4-amines **4a**,**b**,**d**.

Table 2
Denitrosation of *N*,6-disubstituted-*N*-nitrosopyrimidin-4-amines **4a**,**b**,**d**

Starting comp.	R	NR^1_2	Prod.	Prod. nr. in *Tetrahedron* **2007**, 5394.	Yield %
4a	CH_3	$N(CH_2)_4$	**5a**	**8b**	81
4b	$PhCH_2$	$N(CH_2)_4$	**5b**	**8d**	82
4d	$PhCH_2$	$N(CH_2)_4O$	**5c**	**8e**	92

And finally, compound **5c** was treated with an excess of acetic anhydride and triethylformiate under reflux (Scheme 5). The formation of *N*-benzyl-(6-morpholino-4-pyrimidinyl)acetamide **6** took place readily. The product **6** was characterized by the usual spectroscopic methods and by comparing the data with those of authentic sample (**9e**). The IR spectrum of **6** showed strong absorption at 1683 cm^{-1}, which belongs to C=O bond. In the ^1H NMR spectrum, the signals corresponding to the C(2)–H, C(5)–H, and $PhCH_2$ protons appeared as singlets at 8.49, 6.66, and 5.18 ppm, respectively. Besides the multiplets of aromatic protons at 7.23–7.32 ppm and morpholine ring at 3.75–3.79 and 3.57–3.61 ppm, there was a singlet of three protons at 2.25 ppm, which can be attributed to CH_3CO moiety. Interestingly, the latter signal was not noticed in van der Westhuyzen manuscript. In the ^{13}C NMR spectrum of **6** there were signals of 13 inequivalent carbons and the carbon of NCOMe group resonated at 171.4 ppm. The cross-peaks in HSQC spectrum of **6** helped to assign all signals in the ^{13}C NMR spectrum to the corresponding carbons. And finally, from HRMS we found the mass of molecular ion M+H equal to 313.1659 what is in agreement with molecular formula of **6**.



Scheme 5. Acetylation of *N*-benzyl-6-morpholinopyrimidin-4-amine **5c**.

Moreover, compound **5c** did not undergo coupling with 4-chlorophenyldiazonium salt and nitration of C-5 was also unsuccessful. After the nitrosation reaction of **5c**, *N*-nitrosated product **4d** formed.

It is known from the literature and also well-seen from our investigation that introduction of nitrogen containing functional group at the 5-position of insufficiently activated pyrimidines is hardly possible. It would be better to start the synthesis of 2-unsubstituted purines from commercially available 4,6-dichloro-5-nitropyrimidine by classical nucleophilic substitution—reduction of nitro group—cyclization route.[15]

3. Conclusions

In conclusion, since various 5-nitrosopyrimidines are versatile intermediates in organic synthesis, have unique crystal structures and posses notable biological activities; it was our duty to inform the scientific community that direct electrophilic nitrosation reaction of *N*-substituted-6-chloropyrimidin-4-amines is impossible. Instead of electrophilic substitution reaction at the position 5 of the pyrimidine ring, the N-nitrosation of secondary amino group in the 4-position of the pyrimidine ring took place. Moreover we have shown that the synthetic sequence for preparation of purines presented in *Tetrahedron* **2007**, *63*, 5394 by van der Westhuyzen is erroneous.[16]

It may be emphasized that we have conducted all the experiments several times precisely under the reported conditions and found that our results as outlined above are consistently reproducible.

4. Experimental section

4.1. General

IR spectra were run in KBr discs on a Perkin–Elmer FT spectrophotometer Spectrum BX II. ^{1}H and ^{13}C NMR spectra were recorded with a Varian Unity INOVA spectrometer (300 MHz) using residual solvents signals as internal standard. HRMS spectra were obtained on a mass spectrometer Dual-ESI Q-TOF 6520 (Agilent Technologies). All reactions and the purity of the synthesized compounds monitored by TLC using Silica gel 60 F_{254} aluminum plates (Merck). Visualization was accomplished by UV light.

4.2. General method for the N-nitrosation of pyrimidines

A solution of the corresponding 4-aminosubstituted-6-chloropyrimidine (**2a**,**b**) in either acetic acid or hydrochloric acid (2 M) was treated drop-wise with a solution of sodium nitrite (1.8 equiv) in water (6.3 M). The reaction mixture was stirred at room temperature for 2–3 h and a solid precipitate forms over time. The solid is filtered off, washed with water, and dried under suction.

*4.2.1. 6-Chloro-N-methyl-N-nitrosopyrimidin-4-amine (**3a**).* Yield 0.15 g; 88%; yellow powder; mp 78–79 °C. IR (KBr): 3095 (C–H), 1568 (C=N), 1490, 1457, 1166, 1146, 1080, 959, 781 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.86 (1H, s, C(2)–H), 8.06 (1H, s, C(5)–H), 3.46 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.9 (C(4) or C(6)), 161.6 (C(4) or C(6)), 158.1 (C(2)), 107.6 (C(5)), 27.3 (CH_3). HRMS (ES): MH^+, found 173.0244. $C_5H_6^{35}ClN_4O$ requires 173.0230.

*4.2.2. N-Benzyl-6-chloro-N-nitrosopyrimidin-4-amine (**3b**).* Yield 0.22 g; 89%; yellow solid; mp 55–57 °C. IR (KBr): 3092 (C–H), 1568 (C=N), 1568, 1487, 1459, 1392, 1128, 1070, 1059, 984, 927, 888, 777, 728, 711 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.94 (1H, s, C(2)–H), 8.11 (1H, s, C(5)–H), 7.28 (5H, br s, ArH), 5.38 (2H, s, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.9 (C(4) or C(6)), 161.8 (C(4) or C(6)), 158.3 (C(2)), 133.9 (quaternary aryl C), 128.4, 128.1, 127.7 (aryl C), 107.8 (C(5)), 43.4.3 (CH_2). HRMS (ES): MH^+, found 249.0581. $C_{11}H_{10}^{35}ClN_4O$ requires 249.0543.

4.3. General method for the preparation of *N*,6-disubstituted-*N*-nitrosopyrimidin-4-amines (4a–d)

Method A. A mixture of the corresponding 6-chloro-*N*-nitrosopyrimidin-4-amine (**3a**,**b**) in dichloromethane (2 M) was treated drop-wise with 2.2 equiv of the corresponding amine. The mixture was stirred for 18 h at room temperature. Then the resulting solution was washed with water, dried over sodium sulfate, filtered, and concentrated under the reduced pressure affording products **4a**–**d**.

Method B. A mixture of the corresponding 6-chloro-*N*-nitrosopyrimidin-4-amine (**3a**,**b**) in DMF (2 M) was treated drop-wise with 2.2 equiv of the corresponding amine. The mixture was stirred for 10 min at room temperature. After addition of water, precipitated product was filtered, washed with water, and dried overnight.

*4.3.1. N-Methyl-N-nitroso-6-pyrrolidinopyrimidin-4-amine (**4a**).* Yield 0.15 g; 97%; yellowish solid; mp 96–98 °C. IR (KBr): 2973 (C–H), 2868 (C–H), 1598 (C=N), 1552, 1510, 1442, 1442, 1312, 1300, 1183, 1153, 1099, 1021, 960, 819, 729 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.49 (1H, s, C(2)–H), 6.85 (1H, s, C(5)–H), 3.67 (2H, br s, NCH_2), 3.44 (3H, s, CH_3), 3.37 (2H, br s, NCH_2), 2.02 (4H, br s, $(CH_2)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ=160.6 (C(4) or C(6)), 159.6 (C(4) or C(6)), 157.6 (C(2)), 88.3 (C(5)), 46.7 ($(NCH_2)_2$), 27.9 (CH_3), 25.2 ($(CH_2)_2$). HRMS (ES): MH^+, found 208.1195. $C_9H_{14}N_5O$ requires 208.1199.

*4.3.2. N-Benzyl-N-nitroso-6-pyrrolidinopyrimidin-4-amine (**4b**).* Yield 0.185 g; 92%; yellowish needles; mp 105–107 °C. IR (KBr): 2974 (C–H), 2866 (C–H), 1598 (C=N), 1550, 1509, 1475, 1439, 1333, 1319, 1219, 1099, 1078, 1051, 1007, 900, 822, 749, 722, 703 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.57 (1H, s, C(2)–H), 7.28 (5H, br s, ArH), 6.92 (1H, s, C(5)–H), 5.40 (2H, s, PhCH_2), 3.70 (2H, br s, NCH_2), 3.41 (2H, br s, NCH_2), 2.05 (4H, br s, $(CH_2)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.2 (C(4) or C(6)), 159.7 (C(4) or C(6)), 158.1 (C(2)), 135.3 (quaternary aryl C), 128.7, 128.4, 127.7 (aryl C), 88.8 (C(5)), 46.9 ($(NCH_2)_2$), 43.9 (PhCH_2), 25.4 ($(CH_2)_2$). HRMS (ES): MH^+, found 284.1511. $C_{15}H_{18}N_5O$ requires 284.1512.

*4.3.3. N-Methyl-6-morpholino-N-nitrosopyrimidin-4-amine (**4c**).* Yield 0.15 g; 91%; yellowish powder; mp 123–125 °C. IR (KBr): 2980 (C–H), 2877 (C–H), 1596 (C=N), 1550, 1491, 1464, 1447, 1254, 1200, 1176, 1120, 1108, 997, 976, 948, 865, 826, 728 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.52 (1H, s, C(2)–H), 7.11 (1H, s, C(5)–H), 3.76–3.79 (4H, m, $O(CH_2)_2$), 3.66–3.69 (4H, m, $N(CH_2)_2$), 3.43 (3H, s, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ=162.9 (C(4) or C(6)), 160.7 (C(4) or C(6)), 157.6 (C(2)), 87.8 (C(5)), 66.4 ($(OCH_2)_2$), 44.4, ($(NCH_2)_2$), 27.9 (CH_3). HRMS (ES): MH^+, found 224.1109. $C_9H_{14}N_5O_2$ requires 224.1148.

*4.3.4. N-Benzyl-6-morpholino-N-nitrosopyrimidin-4-amine (**4d**).* Yield 0.215 g; 96%; yellowish solid; mp 128–130 °C. IR (KBr): 2868; (C–H), 1594 (C=N), 1549, 1494, 1472, 1445, 1338, 1113, 982, 903, 744, 696 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.61 (1H, s, C(2)–H), 7.29 (5H, br s, ArH), 7.18 (1H, s, C(5)–H), 5.41 (2H, s, PhCH_2), 3.82–3.85 (4H, m, $O(CH_2)_2$), 3.71–3.74 (4H, m, $N(CH_2)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ=162.8 (C(4) or C(6)), 160.2 (C(4) or C(6)), 157.5 (C(2)), 134.6 (quaternary aryl C), 128.2, 127.9, 127.2 (aryl C), 87.7 (C(5)), 66.1 ($(OCH_2)_2$), 44.1, ($(NCH_2)_2$), 43.3 (PhCH_2). HRMS (ES): MH^+, found 300.1459. $C_{15}H_{18}N_5O_2$ requires 300.1461.

4.4. General method for denitrosation of *N*,6-disubstituted-*N*-nitrosopyrimidin-4-amines

To the 3 mL of aqueous sulfuric acid (10%), the corresponding 6,-*N*-disubstituted-*N*-nitrosopyrimidin-4-amine (**4**) was added. The resulting mixture was stirred at 120 °C for 5 min. After cooling to room temperature, the pH of mixture was adjusted to >10 with 2 M aqueous sodium hydroxide. The precipitated solid was filtered off to give pure products **5a**–**c**. Addition amounts of product were obtained by extraction of mother liquid with dichloromethane. It should be noted that the same products were obtained during treatment of reaction mixture with sodium dithionite, as stated in original paper.[10]

*4.4.1. N-Methyl-6-pyrrolidinopyrimidin-4-amine (**5a**).* Yield 0.07 g; 81%; white solid; mp 190–192 °C. IR (KBr): 3243 (NH), 3098 (C–H), 2976 (C–H), 2861 (C–H), 1592 (C=N), 1573, 1539, 1503, 1483, 1439, 1417, 1333, 1301, 1288, 1145, 1099, 973, 795 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.11 (1H, s, C(2)–H), 5.13 (1H, s, C(5)–H), 5.03 (1H, br s, NH), 3.44 (4H, br s, $N(CH_2)_2$), 2.85 (3H, d, J=5.4 Hz, NHCH_3), 1.98 (4H, br s, $(CH_2)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ=162.5 (C(4) or C(6)), 160.5 (C(4) or C(6)), 156.8 (C(2)), 79.4 (C(5)), 46.3 $(NCH_2)_2$, 28.5 (CH_3), 25.3 ($(CH_2)_2$). HRMS (ES): MH^+, found 179.1294. $C_9H_{15}N_4$ requires 179.1297.

*4.4.2. N-Benzyl-6-pyrrolidinopyrimidin-4-amine (**5b**).* Yield 0.105 g; 82%; white powder; mp 169–171 °C. IR (KBr): 3209 (NH), 3071 (C–H), 2962 (C–H), 2858 (C–H), 1591 (C=N), 1530, 1502, 1482, 1444, 1353, 1337, 1279, 1222, 1104, 978, 796, 748, 697 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$): δ=8.12 (1H, s, C(2)–H), 7.33 (5H, br s, ArH), 5.55 (1H, br s, NH), 5.15 (1H, s, C(5)–H), 5.42 (2H, br s, PhCH_2), 3.36 (4H, br s, $N(CH_2)_2$), 1.94 (4H, br s, $(CH_2)_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ=161.9 (C(4) or C(6)), 160.5 (C(4) or C(6)), 157.2 (C(2)), 138.2 (quaternary aryl C), 128.6, 127.3, 127.2 (aryl C), 80.4 (C(5)), 46.2

(NCH_2)$_2$, 45.8 (PhCH_2), 25.2 ((CH_2)$_2$). HRMS (ES): MH^+, found 255.1607. $C_{15}H_{19}N_4$ requires 255.1611.

*4.4.3. N-Benzyl-6-morpholino-N-nitrosopyrimidin-4-amine (**5c**).* Yield 0.125 g; 92%; beige solid; mp 173–175 °C. IR (KBr): 3218 (NH), 2993 (C–H), 2964 (C–H), 1587 (C=N), 1447, 1441, 1333, 1319, 1233, 1204, 1115, 1011, 978, 904, 803, 702 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=8.12 (1H, s, C(2)–H), 7.32 (5H, br s, ArH), 5.54 (1H, br s, NH), 5.38 (1H, s, C(5)–H), 4.44 (2H, d, J=5.7 Hz, PhCH_2), 3.71–3.74 (4H, m, O(CH_2)$_2$), 3.45–3.48 (4H, m, N(CH_2)$_2$). ^{13}C NMR (75 MHz, $CDCl_3$): δ=163.0 (C(4) or C(6)), 162.9 (C(4) or C(6)), 157.4 (C(2)), 138.0 (quaternary aryl C), 127.7, 127.5, 127.2 (aryl C), 80.8 (C(5)), 66.4 ((OCH_2)$_2$), 45.7 (PhCH_2), 44.3, (NCH_2)$_2$. HRMS (ES): MH^+, found 271.1558. $C_{15}H_{19}N_4O$ requires 271.15597.

4.5. Preparation of *N*-benzyl-(6-morpholino-4-pyrimidinyl)-acetamide 6

N-Benzyl-6-morpholino-*N*-nitrosopyrimidin-4-amine (**5c**) (0.07 g, 0.26 mmol) was suspended in a mixture of acetic anhydride (0.35 g) and triethyl orthoformate (0.35 g) and heated to reflux with stirring. After 4 h at reflux, the mixture was evaporated under reduced pressure. The crude residue was purified by flash chromatography using chloroform/ethyl acetate mixture.

Yield 58 mg, 72%; yellowish solid; mp 105–106 °C. IR (KBr): 3034 (C–H), 2965 (C–H), 2857 (C–H), 1683 (C=O), 1577 (C=N), 1482, 1446, 1211, 984, 968 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$): δ=8.49 (1H, s, C(2)–H), 7.22–7.32 (5H, m, ArH), 6.66 (1H, s, C(5)–H), 5.18 (2H, s, PhCH_2), 3.75–3.78 (4H, m, O(CH_2)$_2$), 3.57–3.61 (4H, m, N(CH_2)$_2$), 2.25 (3H, s, COCH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ=171.4 (C=O), 163.0 (C(4) or C(6)), 160.8 (C(4) or C(6)), 157.5 (C(2)), 137.5 (quaternary aryl C), 128.6, 127.3, 127.1 (aryl C), 96.6 (C(5)), 66.3 ((OCH_2)$_2$), 50.2 (PhCH_2), 44.3, (NCH_2)$_2$, 24.2 (COCH_3). HRMS (ES): MH^+, found 313.1659. $C_{17}H_{21}N_4O_2$ requires 313.1666.

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Supplementary data

Supplementary data contains copies of ^1H, ^{13}C NMR and HSQC spectra. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.tet.2012.01.044.

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16. The corresponding author of the original manuscript was contacted by us on 26th of November, 2011; however we did not receive any comments from him about this new evidence.