THE REACTIVITY OF SUBSTITUTED
PURINES IN STRONGLY BASIC MEDIUM

THE OCCURRENCE OF GEOMETRICAL ISOMERISM
IN THE ANIONS OF AROMATIC AMINO COMPOUNDS

Promotor: dr. H.C.van der Plas, hoogleraar in de organische scheikunde

N.J.KOS

THE REACTIVITY OF SUBSTITUTED PURINES IN STRONGLY BASIC MEDIUM

THE OCCURRENCE OF GEOMETRICAL ISOMERISM IN THE ANIONS OF AROMATIC AMINO COMPOUNDS

Proefschrift
ter verkrijging van de graad
van doctor in de landbouwwetenschappen,
op gezag van de rector magnificus,
dr. H.C. van der Plas,
hoogleraar in de organische scheikunde,
in het openbaar te verdedigen
op woensdag 6 mei 1981
des namiddags te vier uur in de aula
van de Landbouwhogeschool te Wageningen

ISN = 13653[illegible]-02

Dit onderzoek werd mede mogelijk gemaakt door financiële steun van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek (Z.W.O.) via de Stichting Scheikundig Onderzoek Nederland (S.O.N.).

STELLINGEN

1

Ondanks de bezwaren verbonden aan andere beschrijvingen, valt het te betreuren dat door Shaw de "oxopurinen" worden voorgesteld als hydroxypurinen met slechts een enkele verwijzing naar hun voorkomen in de amidevorm.

G.Shaw in Rodd's Chemistry of Carbon Compounds, vol.IV, deel L (Ed. S.Coffey), Elsevier (1980).

2

Ter verklaring van de produkten die worden gevormd bij de reactie van arylmercaptanen met epoxiden wordt door Schultz e.a. te weinig waarde aan het stabiliserende effect van de arylthiogroep toegekend.

A.G.Schultz, W.Y.Fu, R.D.Lucci, B.G.Kurr, K.M.Lo en M.Boxer, J.Am.Chem.Soc., 100, 2140 (1978).

3

Het reactiemechanisme dat wordt voorgesteld ter verklaring van het optreden van kleuring wanneer caffeïne met onderchlorigzuur-pyridine wordt behandeld, is aan bedenkingen onderhevig.

K.Kigasawa, K.Ohkubo, T.Kohagisawa, H.Shimizu en S.Saitoh, Heterocycles 4, 1257 (1976).
K.Kigasawa, K.Ohkubo, T.Kohagisawa, H.Shimizu, S.Saitoh en T.Kametani, Yakugaku Zasshi, 97, 18 (1977).

4

Het is uitgesloten dat de vorming van 2,4-diamino-1,6-naftyridine uit 2-amino-4-broom-1,6-naftyridine bij behandeling met kaliumamide in vloeibare ammoniak kan worden verklaard door een eliminatie-additie mechanisme.

W.Czuba, T.Kowalska en P.Kowalski, Polish Journal of Chemistry, 52, 2369 (1978).

5

De structuurformule die Shaw geeft voor herbipoline zou het eerste voorbeeld zijn geweest van een purine waarin zich twee methylgroepen op hetzelfde stikstofatoom bevinden.

G.Shaw in Rodd's Chemistry of Carbon Compounds, vol.IV, deel L (Ed. S.Coffey), Elsevier (1980).

6

De aanwezigheid van een deuteriumatoom op N(7) in N^6, N^6-dimethyladenine hydrochloride kan geen extra belemmering zijn voor rotatie van de dimethylaminogroep.

T.P.Pitner, H.Sternglanz, C.E.Bugg en J.D.Glickson, J.Am.Chem.soc., 97, 885 (1975).

7

Een alternatief mechanisme voor de tele-aminering van 3-broomimidazo [1,2-a] pyridine tot 6-aminoimidazo [1,2-a] pyridine is ook plausibel.

E.Smakula Hand and W.W.Paudler, J.Org.Chem., 43, 2900 (1978).

8

De herkomstomschrijving van Duitse wijnen laat, in tegenstelling tot wat Leenaers beweert, nog wel iets te wensen over.

R.Leenaers, De Duitse Wijnatlas, Keesing (1977).

N.J.Kos

Wageningen, 6 mei 1981

The reactivity of substituted purines in strongly basic medium.
The occurrence of geometrical isomerism in the anions of aromatic amino compounds.

Aan mijn ouders

VOORWOORD

Op deze plaats wil ik graag allen bedanken, die hebben bijgedragen aan het tot stand komen van dit proefschrift.
Mijn promotor, Prof.Dr.H.C. van der Plas, ben ik zeer erkentelijk voor de vele stimulerende discussies, voor zijn snelle en kritische beoordeling van dit manuscript en voor de vrijheid, die hij mij bij dit onderzoek heeft gelaten.
Bij het gebruik van de vele apparatuur, die bij het tegenwoordige chemische onderzoek onmisbaar is, heb ik de steun van velen gehad. In het bijzonder Beb van Veldhuizen heeft zeer veel bijgedragen aan de experimenten, die in dit proefschrift zijn beschreven. Ook de talloze gesprekken met hem waren zeer verhelderend.
Andere waardevolle bijdragen werden geleverd door Willem van Barneveld, Willem Combé, wijlen Job Jansen, Hugo Jongejan, Arie Koudijs, Cees Landheer, Gerrit Lelyveld, Pim Melger, Wim Nieuwboer, Maarten Posthumus en Eef Vleeming.
Bij het chemische werk heb ik erg veel hulp gehad van Wouter Blees.
Bij de uitvoering van het Kokos project heb ik heel prettig samengewerkt met Koos Breuker en heeft Rien van Dijk vele stoffen voor ons aangedragen.
Dit proefschrift kon alleen tot stand komen dankzij het snelle en uitstekende typewerk van Mies Snell en Ruud Hendriksen, het fraaie tekenwerk van Sander Schuchhard en de correcties van Chris Rasmussen.
Tot slot wil ik alle leden van de vakgroep organische chemie bedanken voor de prettige samenwerking.

CONTENTS

Chapters 2, 3 and 4 have been published in the literature

Chapter 2 J.Org.Chem. 44, 3140 (1979).
Chapter 3 Recl.Trav.Chim.Pays-Bas 99, 267 (1980)
Chapter 4 J.Org.Chem. 45, 2942 (1980)

Chapter 6 is in the press

Chapter 6 Heterocycles

Chapters 5 and 7 are submitted for publication

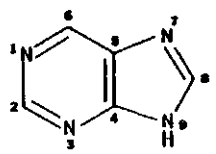
Chapter 5 J.Org.Chem.
Chapter 7 J.Org.Chem.

1 INTRODUCTION

The study presented in this thesis is mainly concerned with reactions of purines in a strongly basic medium. In part A of this introduction a comprehensive review of the nucleophilic substitutions and ring opening reactions of purines will be presented. In addition some electrophilic substitutions in purines will be discussed.
The second part of this thesis describes the occurrence of geometrical isomerism in the anions of aromatic amino compounds. *Aza*-aromatic amino compounds are products of the reactions of *aza* heterocycles with potassium amide in liquid ammonia - the nucleophile used in our study - and show interesting geometrical conformations in this strongly basic medium.
Some aspects of geometrical isomerism, relevant to this subject, will therefore be discussed in section B of this introduction.

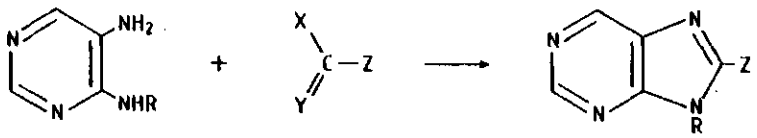
A. Chemistry and history of purines

The chemistry of purines can be considered to have its origin in 1776 with the isolation of uric acid from urinary calculi[1] by both Scheele and Bergmann. Many years passed before the correct empirical and structural formula of the purine skeleton was established.
The final proof for its structure was obtained by Fischer in 1895, showing that the formula proposed by Medicus in 1875 was correct.
The designation "purine" for the ring skeleton, introduced by Fischer, is still in use today (Scheme 1, see reviews).



Scheme 1

In the years thereafter Fischer and co-workers achieved the synthesis of many new purine derivatives, mainly starting from natural purines. A versatile synthesis of purines from other precursors has been developed by Traube (1900). This route, starting from a 4,5-diaminopyrimidine is still the most important method of preparing purines from other precursors (Scheme II).

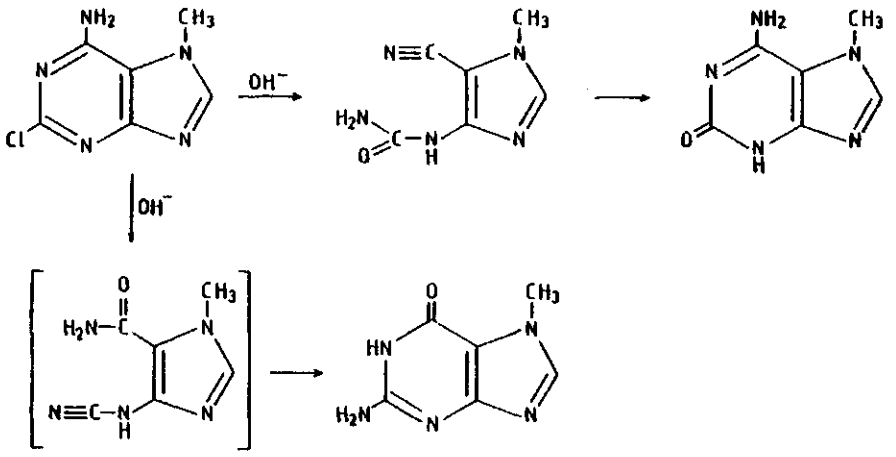


X = OH, Y = O
NH_2 O
Cl O
NH_2 NH

Z = H
C_nH_{2n+1}
OH
SH
NH_2

Scheme 2

Fischer discovered the first rearrangement of the purine ring, when he treated 6-amino-2-chloro-7-methylpurine with base[2]. He obtained 7-methylguanine instead of the expected 7-methylisoguanine. Reinvestigation of this reaction by Shaw showed however, that 7-methylguanine is formed alongside 7-methylisoguanine, indicating that not one but two substitutions take place, both proceeding *via* a ring opening mechanism[3] (Scheme III).



Scheme 3

This reaction can be considered as the first example of a so-called S_N(ANRORC) reaction[4]. This term refers to a reaction that involves a series of steps: Addition of a Nucleophile followed by Ring Opening and Ring Closure. Numerous examples of this mechanism have been found since then in the amide-induced amination of, for example, 2- and 4-halogenopyrimidines[4].
The increasing number of synthetically available purines induced numerous studies on their biological activity. Several effects (diuretic, antiviral, antithyroid) have been reported for purines; the majority of the efforts were directed, however, to increase our knowledge of their antitumor activity.
In 1964 a first review appeared discussing the antitumor activity and structural relationships of purine derivatives[5]. In this field 6-mercaptopurine (leukerin, mercaleukin, purinethol) in particular is still used frequently against acute leukemia.

A.1 Nucleophilic substitutions

Nucleophilic displacements are frequently used for the introduction of substituents and occur easily in purines. In general the reactivity of the purine ring is greatly influenced by deprotonation of the imidazole ring. At position 8 especially a large decrease in reactivity is observed. A discussion of the reactivity of the three different carbon positions has been given by Miller, who showed that in neutral purines the reactivity sequence is $8 \sim 6 > 2$, but in anionic purines $6 > 8 > 2$[6]. A kinetic study of the rate of nucleophilic displacement in the series of 2,6 or 8-(methylthio)-1,3,7 or 9-methylpurines has shown that the position of the methyl group influences the reactivity ($6 > 2 > 8$ in 1-methylpurines and $6 > 8$ in 3-methylpurines)[7].
Several publications show that under controlled conditions gradual substitution is possible. For instance, reaction of 6-chloro-2-fluoropurine with hydroxylamine gives 2-fluoro-N^6-hydroxyadenine at 5°C and 2,6-di(hydroxyamino)purine at reflux temperature[8]. When the nucleophile is present in the side-chain of a purine derivative, nucleophilic displacements can be used for the annellation of rings at the 1,6[9] or 7,8 position[10]. The possibility of introducing a substituted alkyl group at position 6 or 8 *via* a nucleophilic substitution with carbon nucleophiles has stimulated the interest in this type of reactions in recent years. The methylsulfonyl group in particular has been found to be easily replaceable[11-13], as shown by the conversion of 6-(methylsulfonyl)-9-phenylpurine with malononitrile into 9-phenylpurine-6-malononitrile[12].

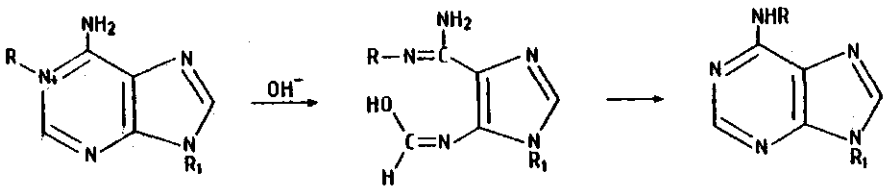
These reactions also occur at position 8 in 8-methylsulfonylpurine nucleosides[11] and at position 6 in 6-chloro-9-phenylpurine[14].
Introduction of an alkyl substituent can also be achieved by Grignard reagents, but the number of applications is limited. 9-Phenylpurine reacts with alkylmagnesiumbromides at position 6, giving 1,6-dihydro-6-alkyl-9-phenylpurine which on oxidation with alkaline ferricyanide yields the corresponding purine[15]. On the other hand 7-phenylpurine undergoes alkylation at position 8, since due to steric hindrance position 6 is blocked[15]. In 8-bromopurine nucleosides[16] the reaction also takes place at position 8. The replacement of hydrogen by an amino group by reaction with alkali amides - the so-called Chichibabin amination - has been stated not to occur with purine[17].

A.2 Ring opening reactions

In the ring opening reactions, that purines undergo, two different patterns can be discerned: i. reactions which involve opening of the pyrimidine ring and give an imidazole derivative, i.i. reactions which lead to opening of the imidazole ring and yield a pyrimidine derivative. Both types of ring opening occur and Badger and Barlin[18] have shown, by comparing the reactivity of 2,6- and 8-monosubstituted 1,3,7- and 9-methylpurines,that the substitution pattern influences the site of nucleophilic attack and determines which of the two rings will be opened.

i. Reactions, involving opening of the pyrimidine ring

The opening of the pyrimidine ring takes place in basic medium and generally involves addition of the hydroxide ion at position 2. The imidazole derivatives can be isolated and used for the synthesis of other compounds, depending on their stability and the reaction conditions. In many cases, however, the substituents present in the initially formed imidazole recyclize under the reaction conditions to give a new purine derivative or other products. The prime example of this type of reactions is the extensively studied Dimroth rearrangement, in which 1-substituted adenines and their salts in basic medium isomerize into an N^6-substituted adenine. Other ring systems, like pyrimidines, can also undergo this rearrangement[19]. The reaction involves addition of the hydroxide ion at position 2, ring opening (*via* cleavage of the N(1) - C(2) bond) and ring closure (Scheme IV).
Recently good evidence for this mechanism has been obtained through the use of ^{15}N-labelled compounds and the isolation of reaction intermediates[20,21].

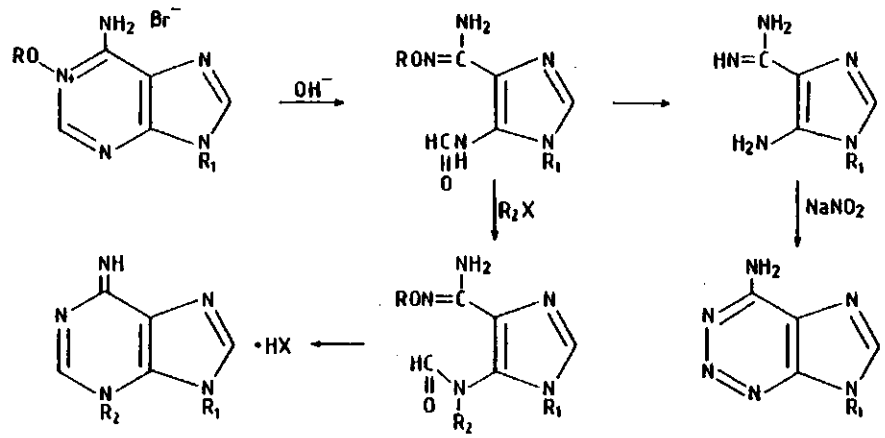


R = CH_2CH_2OH
C_nH_{2n+1}
OC_nH_{2n+1}

R_1 = H
C_nH_{2n+1}
β-D-ribofuranosyl

Scheme IV

The rearrangement of 2-amino-1-methyladenine into 2-methylaminoadenine is another interesting example of such reactions; this reaction, however, involves addition at position 6 and breaking of the N(1) - C(6) bond[22]. The Dimroth rearrangement has also been applied for the synthesis of 2-substituted purines and 2-*aza*purines. For this purpose the reaction intermediate was isolated and reacted with various reagents (Scheme V)[23-26]. 3,9-Dialkyladenines can also be synthesized from these intermediates (Scheme V)[27].



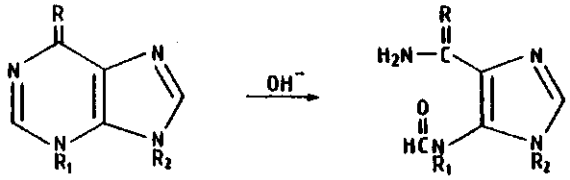
R = C_nH_{2n+1}
$CH_2C_6H_5$

R_1 = C_nH_{2n+1}
$CH_2C_6H_5$
β-D-ribofuranosyl

R_2 = C_nH_{2n+1}
$CH_2C_6H_5$

Scheme V

The previously mentioned Dimroth rearrangements involve the isomerization of 1-mono- and 1,9-disubstituted adenines, but 3,9-disubstituted purines are also vulnerable to attack of the nucleophile at position 2 (Scheme VI)[27-31].



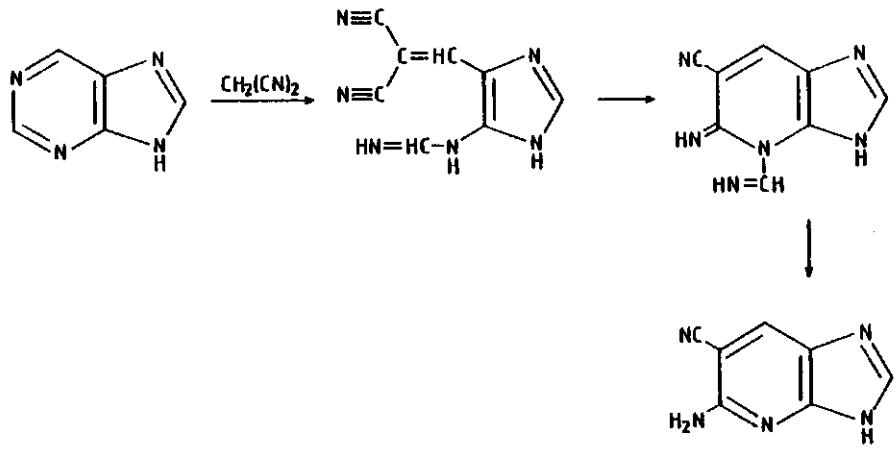
Scheme VI

The cytokinin activity of the N^6 - substituted adenines (plant growth substances) is also observed with 3-substituted adenines. This has been explained by ring opening of the pyrimidine ring and recyclization to N^6 - substituted adenines. Opening of the imidazole ring seems to occur as well, although less readily[32].

Inosine - 1,6-dihydro-6-oxo-9-β-D-ribofuranosylpurine - undergoes attack at position 2 on treatment with aqueous base. This reaction is competitive with isomerization and hydrolysis of the sugar moiety[33]. Remarkably, on reaction with base in the absence of water only hydrolysis occurs[34].

Nucleophiles frequently attack position 6 in purines, but this usually does not lead to ring opening. An interesting exception is the reaction of purine with malononitrile leading to 5-aminoimidazo [4,5-b] pyridine-6-carbonitrile (Scheme VII)[35].

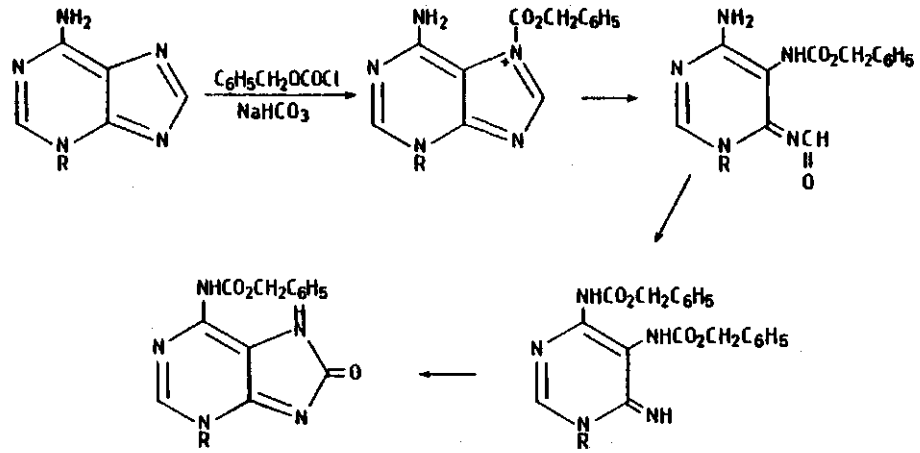
The reaction of 6-methylthiopurine[36], 6-methylthio-9-methylpurine[36] and 1-methyladenine[37] with acids also involves opening of the pyrimidine ring.



Scheme VII

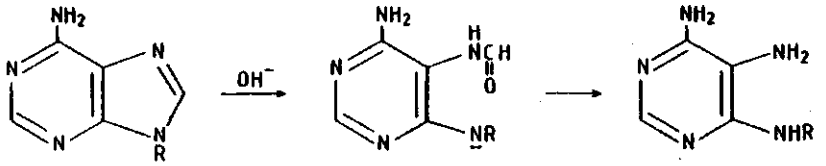
i.i. Reactions involving opening of the imidazole ring

Opening of the imidazole ring is initiated by an attack of the nucleophile at position 8. This is illustrated by the reaction of 3-alkyladenines with carbobenzoxychloride. Initially a 3,7-disubstituted adeninium derivative is formed, which subsequently undergoes nucleophilic attack at position 8, leading to opening of the imidazole ring. Recyclization yields an 8-oxoadenine derivative (Scheme VIII)[38].



Scheme VIII

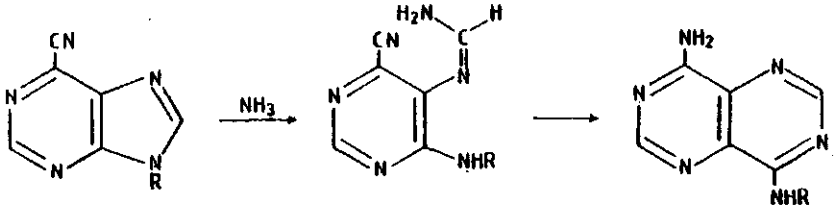
A similar ring opening has also been observed for 7- or 9-substituted purines[39-41]. When the substituent is a sugar moiety the reaction is competitive with cleavage of the glycosidic bond and gives substituted pyrimidines, useful intermediates in the synthesis of 8-*aza*purines (Scheme IX)[42].



R = β-D-ribofuranosyl
2'-deoxy-β-D-ribofuranosyl

Scheme IX

Another example of the vulnerability of the imidazole ring to attack at position 8 is the ring transformation found on treatment of 6-cyano-9-(2',3',5'-tri-O-acetyl-β-D-ribofuranosyl) purine with methanolic ammonia (Scheme X)[43].



R = 2',3',5'-tri-O-acetyl-β-D-ribofuranosyl

Scheme X

A.3 Electrophilic substitutions

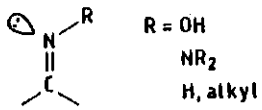
Electrophilic attack on the purine ring only occurs if the ring system is activated by the presence of electron donating groups. These must be present on position 2 or 6 and lead to substitution at position 8. Most classic electrophilic substitutions concern nitration, diazo coupling and halogenation, but more recently electrophilic amination by aromatic hydroxylamines has been found. The amination at C-8 in guanosine by hydroxylamine-O-sulfonic acid may also proceed *via* electrophilic substitution, but this is still uncertain[44].
In the future a renewed interest in electrophilic substitutions may arise from

the possibility that electrophilic attack on purines is important in chemical carcinogenis[45].

A new method of increasing the reactivity of purines toward electrophilic reagents is the formation of an anion at position 6 or 8 *via* a reaction with butyllithium. The resulting anion can react with a variety of electrophiles[46-48]. An example is the formation of 6- or 8-lithio-9-(2'-tetrahydropyranyl) purine from 6-iodo-9-(2'-tetrahydropyranyl) purine with butyllithium. In this reaction the 6-lithio derivative is formed initially and later equilibrates to the 8-lithio derivative. These lithio compounds react with ketones etc. to produce 6- or 8-substituted 9-(2'-tetrahydropyranyl) purines[48].

B. Geometrical isomerism in aromatic amines

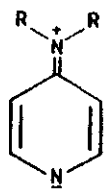
The occurrence of geometrical isomerism in compounds as oximes has been recognized for some time[49].



Scheme XI

This phenomenon has also been observed in butylphenylketimine at temperatures below -40°C in aprotic solvents[50].

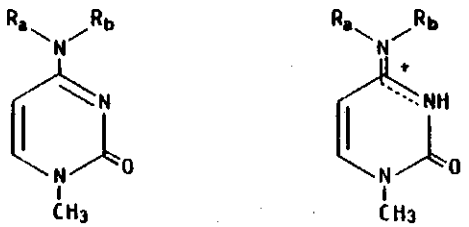
The conjugation between the amino group and the ring in aromatic amines induces a partial double bond character in the C-N bond and brings the substituents on the amino group in the plane of the ring (Scheme XII)[51].



Scheme XII

Geometrical isomerism can be observed in these compounds, providing that, when hydrogen substituents are involved, there is no rapid exchange of the substituents on the amino group. It is remarkable that this condition can even be met

for some N-unsubstituted aromatic amines in water[52]. Several NMR studies have appeared, especially on cytosine derivatives, showing that both the neutral and the cationic compounds (in which the pyrimidine ring is protonated) can occur in two isomeric structures, not only in various organic solvents[53,54] but also in water at a slightly acidic pH[52,55].

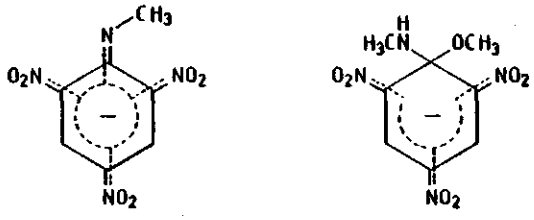


R = H, CH_3

Scheme XIII

Barbieri et al have given a comparison of the rotational barriers in neutral and cationic amines based on dynamic NMR measurements and theoretical calculations[56]. Many aromatic amines show geometrical isomerism at low temperatures. The coalescence temperature depends, of course, on the structure of the compounds and varies considerably: ranging from about -120°C for N-methylanilines[57,58] to above 20°C for 9-substituted 6-methylaminopurines[59].
Rotational barriers are usually determined from dynamic NMR measurements and this method has proved to be more reliable than a method based on ^{15}N chemical shifts[60]. Sterical hindrance and hydrogen bonding between the substituents on the amino group and neighbouring ortho groups are important in determining the isomer ratio and the rotational barrier[59].
It is obvious that any substituent, that influences the conjugation between the amino group and the aromatic ring has a considerable effect on the rotational barrier[58,61-63].
Delocalization of the negative charge leads to conjugation between the deprotonated amino group and the ring in the anions of aromatic amino compounds. However, it is possible that the occurrence of geometrical isomerism in these anions is not observable due to fast exchange in the basic medium, being necessary to generate the anion from the neutral compound.
The NMR spectra of the anions of several anilines[64], aminopyrazine[65] and 2-aminopyridine[65] in liquid ammonia containing potassium amide were reported without mention of the occurrence of geometrical isomerism.

The 2-aminopyridines did not show geometrical isomerism in aprotic solvents as tetrahydrofuran, hexamethylphosphoramide, N,N,N',N'-tetramethylethylenediamine and 1,2-dimethoxyethane containing butyllithium either[66].
The non-equivalency of H-4 and H-6 in the NMR spectrum of the anion of 2-methylaminopyrimidine in liquid ammonia containing potassium amide at -50°C, however, was explained on the basis of geometrical isomerism[67]. This result seems to suggest that the non-occurrence of geometrical isomerism in anilines, aminopyridines and aminopyrazine is due to the fact that the NMR spectra were measured above the coalescence temperature.
Geometrical isomerism was also used as an explanation for the non-equivalency of H-3 and H-5 in the NMR spectrum of N-methyl-2,4,6-trinitroaniline in dimethylsulfoxide containing sodium methoxide[68,69]. This explanation was rejected by Grudtsyn and Gitis, who stated that the NMR spectrum could be explained if a 1:1 σ-adduct between N-methyltrinitroaniline and a methoxide ion is formed[70].



Scheme XIV

A very recent study has shown, however, that the original explanation involving geometrical isomerism in the anion, is the correct one[71].

Reviews

1. J.H.Lister in "The Chemistry of Heterocyclic Compounds. Fused Pyrimidines" Part II. D.J.Brown, Ed., Wiley, New York, 1971.
2. D.T.Hurst. An Introduction to the Chemistry and Biochemistry of Pyrimidines, Purines and Pteridines. Wiley, New York, 1980.
3. G.Shaw in "Rodd's Chemistry of Carbon Compounds", Vol.IV, Part L, S.Coffey, Ed., Elsevier, 1980.

References and Notes

1. It is remarkable that two reviews (reviews 1 and 3) state that uric acid was isolated from gallstones (which contain only very small amounts),while the original publication is entitled "examen chemicum calculi urinarii"
2. E.Fischer, Ber.31, 542 (1898).
3. E.Shaw, J.Org.Chem. 27, 883 (1962).
4. For a review: H.C.van der Plas, Acc.Chem.Res. 11, 462 (1978).
5. R.K.Robins, J.Medicinal Chem. 7, 186 (1964).
6. J.Miller in "Reaction Mechanisms in Organic Chemistry 8 Aromatic Nucleophilic Substitution" C.Eaborn and N.B.Chapman Ed., Elsevier, 1968.
7. R.J.Badger and G.B.Barlin, J.Chem.Soc. Perkin II, 1176 (1976).
8. A. Giner-Sorolla, J.Longley-Cook and J.T.Seg rra, Nucleic Acid Chem. 1, 23 (1978), L.B.Townsend and R.S.Tipson, Ed., Wiley, New York.
9. P.C.Srivastava and R.K.Robins, J.Carbohydr.Nucl. Nucl.4, 93 (1977).
10. M.Eckstein and A.Drabczynska, Synthesis 581 (1979).
11. A.Yamane, Y.Nomoto, A.Matsuda and T.Ueda, Nucleic Acids Res.Spec.Publ. 5, 309 (1978).
12. E.Hayashi and N.Shimada, J.Pharm.Soc.Jap. 99, 201 (1979).
13. A.Yamane, A.Matsuda and T.Ueda, Chem.Pharm.Bull. 28, 150 (1980).
14. E.Hayashi, N.Shimada and K.Watanabe, J.Pharm.Soc.Jap. 99, 205 (1979).
15. E.Hayashi, N.Shimada and Y. Matsuoka, J.Pharm.Soc.Jap. 99, 114 (1979).
16. N.Côngh-Danh, J.P.Beaucourt and L.Pichat, Tetrahedron Lett. 3159 (1979).
17. A.F.Pozharskii, A.M.Simonov and V.M.Doronkin, Russ.Chem.Rev. 47, 1042 (1978).
18. R.J.Badger and G.B.Barlin, J.Chem.Soc.Perkin I 151 (1976).
19. For a review: D.J.Brown in "Mechanisms of Molecular Migrations" 1, 209 (1968). B.S.Thyagarajan Ed., Interscience.
20. G.Grenner and H.L.Schmidt, Chem.Ber. 110, 373 (1977).
21. T.Fujii, T.Itaya and T.Saito, Heterocycles 6, 1627 (1977).
22. G.B.Elion, J.Org.Chem. 27, 2478 (1962).
23. J.A.Montgomery and H.J.Thomas, Nucleic Acid Chem. 2, 681 (1978). L.B.Townsend and R.S.Tipson Ed., Wiley, New York.
24. K.F.Yip and K.C.Tsou, Tetrahedron Lett. 3087 (1973).
25. N.Yamaji, Y.Yasa and M.Kato, Chem.Pharm.Bull. 24, 1561 (1976).
26. N.Yamaji, K.Tahara and M.Kato, Chem.Pharm.Bull. 28, 115 (1980).
27. T.Fujii, T.Saito and M.Kawanishi, Tetrahedron Lett. 5007 (1978).
28. K.Kikugawa, Chem.Pharm.Bull. 25, 2181 (1977).

29. T.Saito and T.Fujii, J.Chem.Soc. Chem.Commun. 135 (1979).
30. T.Fujii, T.Saito and T.Nakasaka, J.Chem.Soc.Chem.Commun. 758 (1980).
31. T.Itaya and H.Matsumoto, Tetrahedron Lett. 4047 (1978).
32. N.J.Leonard and T.R.Henderson, J.Am.Chem.Soc. 97, 4990 (1975).
33. Y.Suzuki, Bull.Chem.Soc.Jap. 47, 898 (1974).
34. H.Follmann, Tetrahedron Lett. 397 (1973).
35. A.Albert and W.Pendergast, J.Chem.Soc.Perkin I 1794 (1973).
36. A.Albert, J.Chem.Soc.C. 2379 (1969).
37. E.R.Garrett and P.J.Mehta, J.Am.Chem.Soc. 94, 8532 (1972).
38. T.Fujii and T.Saito, Chem.Pharm.Bull. 21, 1954 (1973).
39. J.A.Montgomery and C.Temple, J.Am.Chem.Soc. 83, 630 (1961).
40. E.R.Garrett and P.J.Mehta, J.Am.Chem.Soc. 94, 8542 (1972).
41. H.Bredereck, F.Effenberger and G.Rainer, Annalen 673, 82 (1964).
42. J.A.Montgomery and H.J.Thomas, J.Org.Chem. 36, 1962 (1971).
43. H.M.Berman, R.J.Rousseau, R.W.Mancuso, G.P.Kreishman and R.K.Robins, Tetrahedron Lett. 3099 (1973).
44. Y.F.Kawazoe and G.F. Huang, Chem.Pharm.Bull. 20, 2073 (1972).
For a review on the nucleophilic and electrophilic properties of this reagent: Y.Tamura, J.Minamikawa and M.Ikeda, Synthesis 1 (1977).
J.Streith and C.Fizet, Tetrahedron Lett. 3297 (1977).
45. D.E.Hathway and G.F.Kolar, Chem.Soc.Rev. 9, 241 (1980).
46. D.H.R.Barton, C.J.R.Hedgecock, E.Lederer and W.B.Motherwell, Tetrahedron Lett. 279 (1979).
47. M.Côngh-Danh, J.P.Beaucourt and L.Pichat, Tetrahedron Lett. 2385 (1979).
48. N.J.Leonard and J.D.Bryant, J.Org.Chem. 44, 4612 (1979).
49. C.G. McCarty in "The chemistry of the carbon-nitrogen double bond", S.Patai Ed., Interscience, 1970.
50. J.B.Lambert, W.L.Oliver and J.D.Roberts, J.Am.Chem.Soc. 87, 5085 (1965).
51. H.Kessler, Angew.Chem. 82, 237 (1970).
52. B.McConnell and P.C.Seawell, Biochemistry 12, 4426 (1973).
53. R.R.Shoup, H.T.Miles and E.D.Becker, J.Phys.Chem. 76, 64 (1972).
54. E.D.Becker, H.T.Miles and R.B.Bradley, J.Am.Chem.Soc. 87, 5575 (1965).
55. M.J.Raszka, Biochemistry 13, 4616 (1974).
56. G.Barbieri, R.Benassi, R.Grandi, U.M.Pagnoni and F.Taddei, J.Chem.Soc. Perkin II 330 (1979).
57. L.Lunazzi, C.Magagnoli, M.Guerra and D.Macciantelli, Tetrahedron Lett. 3031 (1979).

58. L.Lunazzi, C.Magagnoli and D.Macciantelli, J.Chem.Soc.Perkin II 1704 (1980)
59. G.Dodin, M.Dreyfus and J.E.Dubois, J.Chem.Soc.Perkin II 438 (1979).
60. F.A.L.Anet and M.Ghiaci, J.Am.Chem.Soc. 101, 6857 (1979).
61. J.Almog, A.Y.Meyer and H.Shanan-Atidi, J.Chem.Soc.Perkin II 451 (1972).
62. J.Riand, M.T.Chenon and N.Lumbroso-Bader, J.Chem.Soc.Perkin II 1248 (1979).
63. M.L.Filleux-Blanchard, J. Fieus and J.C.Hallé, Org.Magn.Reson. 5, 221 (1973).
64. T.Birchall and W.L.Jolly, J.Am.Chem.Soc. 88, 5439 (1966).
65. J.A.Zoltewicz and L.S.Helmick, J.Org.Chem. 38, 658 (1973).
66. K.Konishi and K.Takahashi, Bull.Chem.Soc.Jap. 50, 2512 (1977).
67. J.P.Geerts, H.C.van der Plas and A.van Veldhuizen, Org.Magn.Reson. 7, 86 (1975).
68. K.L.Servis, J.Am.Chem.Soc. 87, 5495 (1965).
69. K.L.Servis, J.Am.Chem.Soc. 89, 1508 (1967).
70. Yu.D.Grudtsyn and S.S.Gitis, J.Org.Chem.USSR 11, 2616 (1975).
71. M.R.Crampton, B.Gibson and R.S.Matthews, Org.Magn.Reson 13, 455 (1980).

2 THE CHICHIBABIN REACTION OF PURINES WITH POTASSIUM AMIDE IN LIQUID AMMONIA

Nico J. Kos, Henk C. van der Plas and Beb van Veldhuizen

Reaction of purine, 2-methyl- and 8-methylpurine with potassium amide in liquid ammonia leads to the formation of adenine, 2-methyl- and 8-methyladenine respectively. 6-Methyl- and 6,8-di-tert-butylpurine do not react. It was proven by applying ^{15}N-labelled potassium amide, that the amination reactions do not involve opening of the pyrimidine ring. Low temperature NMR spectroscopy showed that in solutions of purine and 2-methylpurine in potassium amide - liquid ammonia an anionic σ-complex at position 6 is formed. 8-Methylpurine on the contrary only showed the presence of a monoanion and a dianion.

Introduction

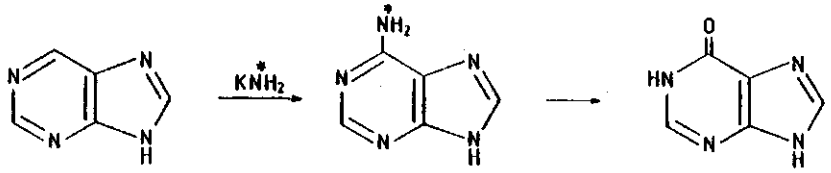
It is well known that purines are in general more susceptible to nucleophilic than to electrophilic attack.[2] In basic medium, however, the reactivity towards nucleophiles is often strongly decreased due to deprotonation of the NH of the imidazole ring.[2] Deprotonation has as further consequence that the pattern of addition of nucleophiles changes. Whereas in neutral purines both positions 6 and 8 are reactive in nucleophilic additions, in the anions of purines addition to position 8 is found to be prohibited (due to Coulomb repulsion) and addition takes place only at position 6.[2] In both neutral and anionic purines position 2 is the least reactive.[2] The interesting fact, observed for the first time in our laboratory, that pyrimidines[3] and s-triazines[4] can undergo Chichibabin amination[5] with potassium amide in liquid ammonia involving partly a ring-opening, ring-closure reaction sequence S_N(ANRORC-mechanism) [6], induced us to investigate in detail the behaviour of purine and of the three isomeric C-methylpurines towards the same reagent.

Results and Discussion

Amination of purine

When purine (1) reacts with 4 equivalents of potassium amide in liquid ammonia,

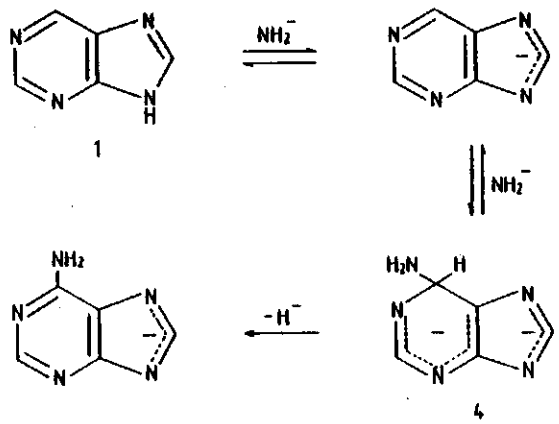
adenine (2) is formed as sole product. The reaction rate of this Chichibabin amination is very low; after 20 h 30% of purine can still be recovered. However, after 70 h the conversion of 1 into 2 is quantitative. This method of preparation of 2 is new and till now unexplored. The reaction is remarkable since under the conditions of the reaction 1 is certainly present in its anionic form (pK_a purine: 8.9)[7] but despite of that, a nucleophilic attack by the amide ion on this negatively charged species can take place. When the amination was carried out with ^{15}N-labelled potassium amide we found that in the labelled adenine (2)* the label was present exclusively in the nitrogen of the amino group, no trace of ^{15}N-label was present in the nitrogen atoms of the ring. This was proven by conversion of 2* into hypoxanthine (3) by diazotization[8] and by determining the ^{15}N-content in 2* (% ^{15}N: 10.9 (7.6)[9]) and 3 (% ^{15}N: 0.0 (0.0)[9]) using mass spectrometry.



1 2* 3

From these results it is evident that in the formation of 2 from 1 no S_N(ANRORC)-mechanism [6], is involved but that the amination follows a pathway in which addition of the amide ion takes place at position 6, followed by loss of a hydride ion. This is in agreement with the general observation that in the anion of purine position 6 is the most reactive position for the addition of nucleophiles.[2,10]

The formation of adenine from the anion of 1 raises the interesting question, which step in the amination is rate-determining.[11] Two possibilities can be considered. The first one is that the addition of the negatively charged amide ion to position 6 of the anion of 1 is rate-determining. It leads to dianion 4 as intermediate σ-adduct. The second possibility -also reasonable- is that the aromatization step yielding the monoanion of adenine is rate determining. Our first attempt to tackle this problem was to measure the influence of deuterium at position 6 on the rate of amination. A competition experiment between purine and 6-deuteropurine however, met with little success, since 6-deuteropurine was found to undergo a slow D/H exchange under the reaction



conditions. An attempt to detect the intermediate 4 by ^{1}H- and ^{13}C-NMR spectroscopy was more successful. When 1 was dissolved in liquid ammonia containing potassium amide (0.07 - 0.5 mmol of 1 in 1 ml of liquid ammonia containing 4 - 10 equivalents of potassium amide) the ^{1}H- and ^{13}C-NMR spectra showed first the formation of the anion of 1. Then the anion is slowly converted into a σ-adduct as indicated by a strong upfield shift of 3.04 ppm for one of the hydrogens and 76.1 ppm for one of the carbon atoms (change of hybridization of $sp^2 \rightarrow sp^3$) (see Table I and II). The appearance of signals of the adduct leads at the same time to a disappearance of the signals of the anion of 1. The upfield shift values are in good agreement with those reported in the literature.[10,13,14] No splitting of the proton signal at δ 5.75 into a triplet was observed due to a fast hydrogen exchange[13] caused by the large excess of potassium amide. That the formation of the adduct takes place at position 6 was unequivocally proven by comparing the ^{1}H- and ^{13}C-NMR spectra of 6- and 8-deuteropurine in liquid ammonia containing potassium amide (Table I and II). These results strongly indicate that the second step in the amination, the aromatization step is thus the rate-determining step. The data further prove that addition of the amide ion to the anion of purine is easily possible.[15]

6-methylpurine

In order to investigate whether the presence of a substituent at position 6 would lead to amination at another position, we investigated 6-methyl- (5) and 6,8-di-t-butylpurine. It can be expected that the amination of methylpurines by potassium amide will be strongly retarded due to formation of a dianion through

Table I *^{1}H-NMR Data (δ Values) of Purines. Methylpurines and its several Deutero Derivatives in Liquid Ammonia containing Potassium Amide*

		H-2	H-6	H-8	$CH_3(CH_2^-)$
purine	anion	8.58	8.79	8.12	
	adduct	6.95	5.75	6.83	
6-D-purine	anion	8.62		8.15	
	adduct	6.96		6.85	
8-D-purine	anion	8.61	8.82		
	adduct	6.99	5.76		
6-CH_3-purine	anion	8.48		8.06	2.68
	dianion	7.24		7.09	3.02[Ψ]
6-CH_3-8-D-purine	anion	8.48			2.68
	dianion	7.21			3.01[Ψ]
2-CH_3-purine	anion		8.68	8.05	2.60
	adduct		5.69	6.83	1.77
2-CH_3-8-D-purine	anion		8.71		2.63
	adduct		5.71		1.80
8-CH_3-purine	anion	8.42	8.54		2.48
	dianion	7.35	6.77		2.48
6-D-8-CH_3-purine	anion	8.40			2.44
	dianion	7.29			2.44
6,8-di-t-butylpurine	neutral ($CDCl_3$)	9.00			1.61 and 1.65

[Ψ]AB quartet (J = 3 Hz)

proton abstraction of both the NH and the methyl group. We found that the reaction of 5 with potassium amide in liquid ammonia for 140 h did not give a product. An ^{1}H-NMR spectrum of this solution first showed the formation of anion (6), which, with a larger excess of potassium amide, was completely converted into the dianion 7 as illustrated by the appearance of the CH_2^- signal as an AB quartet[16] (Scheme III, Table I).

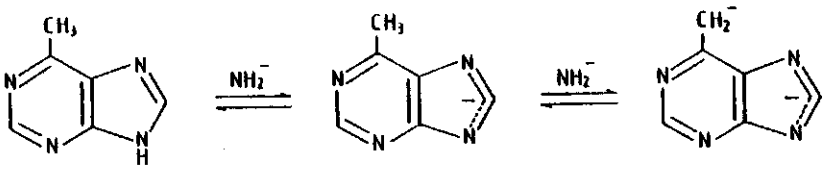
Table II *^{13}C-NMR Data of Purines and Reaction Intermediates in DMSO-d-6 and Liquid Ammonia containing Potassium Amide*

		C-2	C-4	C-5	C-6	C-8	$CH_3(CH_2^-)$
purine	neutral[12]	152.2	154.7	130.5	145.6	146.1	
	anion[12a]	149.5	160.7	134.5	143.7	156.9	
	adduct	153.6	147.5	118.3	67.6	138.9	
2-CH_3-purine	neutral	161.0	155.0	128.8	145.3	145.3	25.5
	anion	156.7	164.5	134.5	143.3	158.4	25.7
	adduct	158.4	149.3	116.7	67.7	138.8	27.0
8-CH_3-purine	neutral	151.5	155.9	131.2	143.3	156.2	15.1
	anion	148.4	165.2	138.5	140.7	168.2	18.6
	dianion	143.8	*	*	115.8	*	47.5
6-NH_2-8-CH_3-purine	neutral	151.6	***	***	***	***	14.6
6,8-di-t-butylpurine**	neutral ($CDCl_3$)	149.7	153.8	132.5	163.0	168.5	

* These data could not be obtained due to slow decomposition with the large excess of potassium amide. It was therefore also very difficult to obtain ^{13}C-NMR spectra of 6-methylpurine in liquid ammonia with potassium amide.

** The signals of 6- and 8-t-butyl groups appear at 38.6 and 29.4 ppm and 34.1 and 29.3 ppm respectively. This shows that one of the t-butyl groups must be at position 8 (H.C.van der Plas and A.Koudijs, unpublished results). The coupling constant for C-2 (J_{C-H}= 202 Hz) further shows that the other t-butyl group must be at position 6 (see ref 17).

*** These data could not be obtained due to the very low solubility of this compound, however the position of the two signals and the coupling constant for C-2 (J_{C-H}= 197 Hz) show that this structure must be correct.

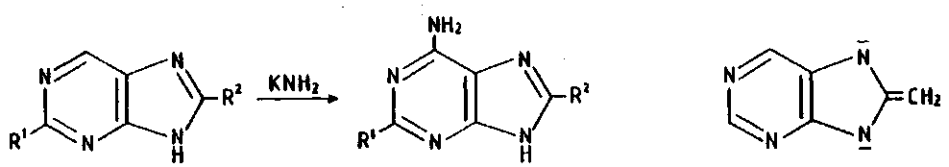


5 6 7

The ^{1}H-NMR signals were assigned by comparison with those of 6-methyl-8-deuteropurine. 6,8-Di-t-butylpurine, from which only a monoanion and no dianion can be formed, was found to be completely inactive. These experiments show that blocking of position 6 in the purine ring does not lead to another position for nucleophilic attack!

2-Methylpurine

When 2-methylpurine (8a) is reacted with potassium amide in liquid ammonia for 70 h a tarry mass is obtained from which 2-methyladenine (9a, 20%) can be isolated; 5% of 8a can be recovered. The structure of 9a was proven by mass spectroscopy and comparison of the ^{13}C-NMR spectrum[17] and the UV spectrum[18] with those reported in the literature. The ^{1}H- and ^{13}C-NMR spectra of solutions of 8a in liquid ammonia containing potassium amide showed the presence of an adduct at position 6 as shown by the upfield shift of 2.94 ppm for H-6 and 77.6 ppm for C-6. This adduct was slowly formed from the anion of 2-methylpurine (Table I and II). The assignment of the ^{1}H-signals was unequivocally established by comparison with the signals of 2-methyl-8-deuteropurine. We found no spectroscopic evidence for the presence of a dianion, although this does not exclude the presence of this species in a very low concentration.



8 9 10

a. $R^1 = CH_3$; $R^2 = H$
b. $R^1 = H$; $R^2 = CH_3$

8-Methylpurine

After reacting 8-methylpurine (8b) with potassium amide for 70 h a tarry mass was obtained; we recovered 25% of 8b and isolated 8-methyladenine (9b) in 25% yield. The structure of this product was confirmed by mass spectroscopy, ^{13}C-NMR spectroscopy and by comparison of the UV spectrum with that reported in the literature.[19] Reaction with ^{15}N-labelled potassium amide gave ^{15}N-labelled 8-methyladenine (% ^{15}N: 7.4 (7.1)[9]). Diazotization[8] yielded unlabelled 8-methylhypoxanthine (% ^{15}N 0.0 (0.0)[9]) showing that the formation of 9b from 8b does not proceed via a ring opening reaction. Compound 8b thus reacts identically as purine towards potassium amide. Attempts to obtain spectroscopic evidence for the intermediacy of a σ-adduct failed. The ^{1}H- and ^{13}C-NMR spectrum of a solution of 8b in liquid ammonia containing potassium amide showed besides the monoanion only the presence of a dianion. This is clearly indicated by the small upfield shift observed: 1.07 ppm for H-2, 1.77 ppm for H-6 and 24.9 ppm for C-6 (Table I and II). The ^{13}C signal of the side chain carbon was split into a triplet indicating the presence of a CH_2^- group. However in the ^{1}H-NMR spectrum the signal of the hydrogens of the side chain at δ 2.48 appeared as a singlet instead of an AB quartet as observed for the signal of the CH_2^- group in 6-methyladenine. This is certainly due to the fact that both hydrogens are in a symmetrical chemical environment towards the imidazole ring (see formula 10). It is unlikely that this dianion could be an intermediate in the amination -in case addition of an amide ion would take place it gives a trianion- it seems therefore more likely that an adduct between a monoanion and the amide ion must be present in the solution, although in a concentration too low to be detected by NMR spectroscopy. We observed that 6-deutero-8-methylpurine undergoes a rapid D/H exchange during the NMR measurements. This behaviour is in remarkable contrast to the very slow D/H exchange observed with 6-deuteropurine.

Experimental Section

^{13}C- and ^{1}H-NMR spectra were obtained with a Varian XL-100-15 spectrometer, equipped with a Varian 620/L16K computer. ^{1}H spectra were recorded also on a Jeol C-60H spectrometer, equipped with a JES-VT-3 variable temperature controller. When measuring in DMSO d-6 internal TMS was used as standard. When measuring in liquid ammonia the sample temperature was ca. -50°C. For ^{13}C-NMR spectra trimethylamine was used as internal standard. These spectra were converted

to the TMS scale by adding 47.5 ppm. Typical ^{13}C spectral parameters were as follows: spectral width 5120 Hz, acquisition time 0.8 s, pulse delay 1.2 s, pulse width 10 µs. For ^{1}H spectra NH_3 was used as standard. The spectra were converted to the TMS scale by adding 0.95 ppm. Mass spectra and ^{15}N contents were determined on an AEI MS-902 mass spectrometer. IR spectra were obtained with a Perkin Elmer 237 and an Hitachi EPI-G3 and UV spectra with a Beckman Acta CIII.

Preparation of Starting Materials

Purine[20], *2-methyl-*[21] *and 8-methylpurine*[22] were prepared as described in the literature. 6-Methylpurine was purchased from Sigma and adenine from Merck. ^{15}N-labelled ammonia was prepared by reacting ^{15}N-labelled ammonium nitrate (from VEB Berlin-Chemie) with potassium hydroxide.

6,8-Di-t-butylpurine. 1.3 G of purine, 0.15 g of silver nitrate and 6.5 g of pivalic acid were dissolved in a solution of 1 g of sulfuric acid in 10 ml of water.[23] With stirring 10 g of ammoniumperoxydisulfate dissolved in 30 ml of water were added in 45 minutes, followed by an additional 45 minutes stirring. The solution was made alkaline with aqueous sodium hydroxide and extracted with chloroform. The extracts were dried ($MgSO_4$), the solvent was evaporated and the residue purified by column chromatography on silica gel using chloroform /ethylacetate 1:1 as eluent, followed by sublimation in vacuo (160 - 180°C at 15 mm) and recrystallization from hexane; yield 1.7 g (67%), mp 194 - 195°C. Anal. Calcd for $C_{13}H_{20}N_4$ C,67.20; H,8.68. Found C,67.50; H,8.81. The structure was proven by ^{1}H- and ^{13}C-NMR spectroscopy (see Table I and II).

8-Deutero- and 2-methyl-8-deuteropurine were obtained by refluxing purine and 2-methylpurine respectively in deuteriumoxide.[24]

6-Deutero-8-methylpurine was prepared by the same method.[24] The position of deuteration was proven by NMR spectroscopy.[25]

6-Deuteropurine was prepared by introducing oxygen in a solution of 6-hydrazinopurine (prepared from 6-chloropurine)[26] in deuteriumoxide containing sodium hydroxide.[27] For NMR measurements the deuterium labelled compounds were diluted to about 50% deuterium content.

Reactions with Potassium Amide. 1 Mmol purine was reacted with 4 mmol potassium amide dissolved in 15 ml dry liquid ammonia. After 20 or 70 h the reaction was quenched with ammoniumsulfate, the ammonia evaporated and the residue extracted with methanol. Separation of the products was achieved by column chromatography or preparative TLC with mixtures of methanol and chloroform as eluent.

Acknowledgement

This investigation was carried out under the auspices of the Netherlands Foundations for Chemical Research (SON) and with financial aid from the Netherlands Organisation for Advancement of Pure Research (ZWO). We are indebted to Drs. C. A. Landheer and Mr. W. P. Combé for the mass spectroscopy data, Mr. A. Koudijs for the synthesis of 6,8-di-t-butylpurine and Mr. H. Jongejan for carrying out the microanalysis.

References and Notes

(1) Part LXXVII on pyrimidines from this laboratory. For part LXXVI see D.A.de Bie, A.Nagel, H.C.van der Plas, G.Geurtsen and A.Koudijs, Tetrahedron Letters, 649 (1979).
(2) For a review: J. H. Lister in The Chemistry of Heterocyclic compounds. Fused Pyrimidines part II ed. D.J.Brown (1971).
(3) C.A.H.Rasmussen and H.C.van der Plas, Recl. Trav. Chim., 97, 288 (1978).
(4) Gy.Simig and H.C.van der Plas, Recl. Trav. Chim., 95, 113 and 125 (1976).
(5) The term "Chichibabin amination" refers to a reaction in which the hydrogen in an aromatic heterocycle is replaced by an amino group.
(6) The term S_N(ANRORC) mechanism (Addition of the Nucleophile, Ring Opening and Ring Closure) refers to a reaction in which an opening of the heterocyclic ring occurs. For a review: H.C.van der Plas, Acc. of Chem. Res., 11, 462 (1978).
(7) S.Lewin and M.A.Barnes, J. Chem. Soc. B 478 (1966).
(8) M.Krüger, Z. Physiol. Chem., 18, 444 (1886).
(9) The number in brackets refers to a duplicate experiment; the accuracy of the mass spectrometric determinations is ± 0,2%.
(10) A σ-adduct on position 6 has been found for purine and some derivatives with barbituric acid and 2-thiobarbituric acid and potassium hydrosulfite (W.Pendergast, J. Chem. Soc. P I, 2758 (1973) and 2240 (1975)) but covalent hydration has never been found (A.Albert, J. Chem. Soc., B 438 (1966)).

(11) Studies on the Chichibabin amination in pyridines have shown that the second step i.e. the aromatization step is considered rate determining (S. V.Kessar, U.K.Nadir and Manmohan Singh, Indian J. Chem., 11, 826 (1973)). This is also shown by the observation of an intermediary σ-adduct with triazine- and pyrimidine derivatives (see ref 3 and 4).

(12) E.Breitmaier and W.Voelter, Tetrahedron 30, 3941 (1974).

(12a)R.J.Pugmire and D.M.Grant, J. Am. Chem. Soc., 93, 1880 (1971).

(13) J.P.Geerts, C.A.H.Rasmussen, H.C.van der Plas and A.van Veldhuizen, Recl. Trav. Chim., 93, 231 (1974).

(14) J.P.Geerts, H.C.van der Plas and A.van Veldhuizen, Org. Magn. Res., 7, 86 (1975).

(15) This has also been found in 4-methyl(amino)-5-bromopyrimidines. In these compounds a proton is abstracted from the substituent to form an anion, but in that case the formation of a σ-adduct could not be shown (C.A.H. Rasmussen, H.C.van der Plas, P.Grotenhuis and A.Koudijs, J. Het. Chem., 15, 1121 (1978)).

(16) 4-Methyl- and 4-methyl-5-bromopyrimidine (see ref 13) and 2-methylpyridine (J.A.Zoltewicz and L.S.Helmick, J. Org. Chem., 38, 658 (1973)) show a splitting of the methylene group into an AB pair of doublets (J = 2.3 Hz) when dissolved in liquid ammonia, containing potassium amide. This is due to the rescinding of free rotation and the resulting difference in chemical environment of the methylene protons.

(17) M.C.Thorpe and W.C.Coburn, J. Magn. Res. 15, 98 (1974).

(18) E.C.Taylor, O.Vogel and C.C.Cheng, J. Am. Chem. Soc., 81, 2442 (1959). J. Baddiley, B.Lythgoe and A.R.Todd, J. Chem. Soc., 318 (1944).

(19) We suggest that the values reported in the literature (λ_{max} 269 nm at pH1 and 266 nm at pH10) were interchanged. We found the same values of λ_{max} but interchanged at the diffent pH's, thus λ_{max} 266 nm at pH1 and 269 nm at pH10). All related compounds (purine λ_{max} 260 nm at pH0.3 and λ_{max} 271 nm at pH11, 2-methylpurine λ_{max} 266 at pH1 and λ_{max} 275 nm at pH11, 8-methylpurine λ_{max} 264 nm at pH0 and λ_{max} 274 nm at pH12, adenine λ_{max} 263 nm at pH2 and λ_{max} 269 nm at pH12, 2-methyladenine λ_{max} 266 nm at pH1 and λ_{max} 271 nm at pH13 and 2,8-dimethyladenine λ_{max} 269 nm at pH1 and λ_{max} 271 nm at pH11) show that the λ_{max} at pH1 is always lower than at pH10 (H.C.Koppel and R.K.Robins, J. Org. Chem., 23, 1457 (1958). See also ref 2).

(20) J.A.Montgomery and C.Temple, J. Org. Chem., 25, 395 (1960).

(21) R.N.Prasad, C.W.Noell and R.K.Robins, J. Am. Chem. Soc., 81, 193 (1959). R.K.Robins, J.W.Jones and H.H.Lin, J. Org. Chem., 21, 695 (1965).
(22) A.Albert and D.J.Brown, J. Chem. Soc., 2060 (1954).
(23) We applied the same procedure as described before. See H.C.van der Plas and A.Koudijs, Recl. Trav. Chim., 97, 159 (1978).
(24) R.J.Pugmire, D.M.Grant, R.K.Robins and G.W.Rhodes, J. Am. Chem. Soc., 87, 2225 (1965).
(25) S.Matsuura and T.Goto, J. Chem. Soc., 623 (1965).
(26) This reaction was carried out with hydrazine hydrate instead of anhydrous hydrazine (J.A.Montgomery and L.B.Holum, J. Am. Chem. Soc., 79, 2185 (1957)).
(27) C.Temple, C.L.Kussner and J.A.Montgomery, J. Org. Chem., 30, 3601 (1965).

3 THE BEHAVIOUR OF 6- AND 8-SUBSTITUTED PURINES TOWARD POTASSIUM AMIDE IN LIQUID AMMONIA

Nico J. Kos, Henk C. van der Plas and Beb van Veldhuizen

Reaction of 6-chloro- and 6-(methylthio)purine with potassium amide in liquid ammonia leads to the formation of adenine. When these aminations are carried out with ^{15}N-labelled potassium amide, the ^{15}N-label is found to be present in the amino group, proving that these reactions do not involve opening of the pyrimidine ring. Low temperature ^{1}H- and ^{13}C-NMR spectroscopy of solutions of 6-chloro- and 6-(methylthio)purine in liquid ammonia, containing potassium amide give no evidence for the presence of an intermediary σ-adduct. 8-Chloro- and 8-(methylthio)purine undergo a Chichibabin amination at position 6 leading to 8-chloro-and 8-(methylthio)adenine. In addition 8-chloropurine gives adenine. Evidence is presented that the formation of this product proceeds via a tele-amination. 6-tert-Butyl-8-(methylthio)purine and 8-aminopurine are found to be unreactive towards potassium amide.

Introduction

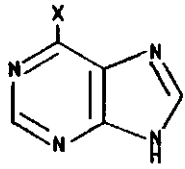
Purines are in general very susceptible to nucleophilic attack. Under basic conditions this reactivity is considerably decreased due to deprotonation of the NH group of the imidazole ring.[2] However we recently reported that with the strong nucleophile and base potassium amide purine and some of its C-methyl derivatives undergo amination to adenine and methyladenines respectively.[3] It seems of interest to study also the behaviour of purines containing a leaving group, with potassium amide in liquid ammonia. In this paper we report on the reactions of purines, containing a chloro or methylthio group at position 6 or 8. A systematic study of the reactivity of the monochloropurines with sodium ethoxide and piperidine has already been published.[4]

Results and discussion

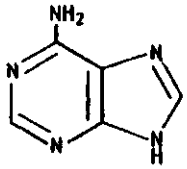
Amination of 6-methylthio- and 6-chloropurine

When 6-(methylthio)purine (1a) or 6-chloropurine (1b) reacts with 4 equivalents

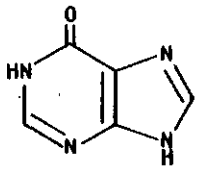
of potassium amide in liquid ammonia at -33°C for 20 h adenine (2) is formed in a nearly quantitative yield. By comparison, the reaction of 1b with ammonia in butanol requires 10 h at 150°C.[6]



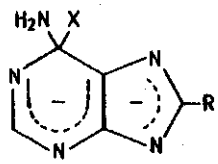
1a, X = SCH_3
b, X = Cl



2



3



4

Since both compounds 1a and 1b will certainly be present as anion under the applied reaction conditions pK_a (1a) = 8.8[5] and pK_a (1b) = 7.8,[5,6] the nucleophilic attack of the amide ion will take place on the anionic species[3]. When the amination reactions are carried out with ^{15}N-labelled potassium amide we found labelled adenine (2*) in which the label was present exclusively in the nitrogen of the amino group. This was proven by conversion of 2* into hypoxanthine (3) by diazotization[7] and by determining the ^{15}N-content in 2* and 3 (Table I). From these results it is evident that in the formation of 2 from 1a and from 1b no S_N(ANRORC) mechanism[8] is involved. The amination probably occurs according to an S_N(AE) mechanism, involving as intermediate the highly reactive dianionic σ-adduct 4 (X = SCH_3, Cl; R = H). These results are in agreement with the observation that position 6 is the most reactive position for nucleophilic attack in anionic purines.[2,3] Attempts to detect the intermediate 4 by NMR spectroscopy failed. When 1a or 1b was dissolved in liquid ammonia containing potassium amide (0.3 - 0.5 mmol of 1a or 1b in 1 ml of liquid ammonia containing 4 - 10 equivalents of potassium amide) the ^1H- and ^{13}C NMR spectra showed first the formation of the anion of 1a or 1b. Then the anion slowly disappears as the reaction proceeds. In the spectra the formation of the dianion of 2 is then visible (Table II and III). It is remarkable that the ^1H- and ^{13}C NMR spectra of solutions of adenine (2) in liquid ammonia, containing potassium amide show that the dianion of this compound is present in two isomeric forms; also the -NH proton can be observed in ^1H NMR (δ = 4.59 and 4.93) as two distinctive signals. This is probably due to a restricted rotation around the C(6)-NH bond. The generality of this phenomenon will be discussed in more detail in a forthcoming publication. In the case of 1a a signal for the methylsulfide anion at δ = 1.78 (formed during the formation of 2) also appears. The ^1H signals could definitively be

Table I ^{15}N *contents (percentages) of the products obtained in the reaction of the starting compounds with* ^{15}N*-labelled potassium amide*

products / starting compound	adenine (2)	hypoxanthine (3)	8-SCH_3-adenine (6a)	8-SCH_3-hypoxanthine
6-SCH_3-purine (1a)	9.1	0.3	-	-
	6.3	0.2	-	-
6-Cl-purine (1b)	9.8	0.0	-	-
	5.6	0.1	-	-
8-SCH_3-purine (5a)	-	-	4.6	0.1
	-	-	4.3	0.0
8-Cl-purine (5b)	4.5	0.2	-	-
	6.7	0.1	-	-

The accuracy of the measurements is $\pm$ 0.2%

assigned by measuring the 8-deuterio derivatives of 1a, 1b and 2. The ^{13}C NMR absorptions were assigned by comparison of the spectra with those of the neutral compounds and the anion of purine.[3] It is of interest to note that in the KNH_2/NH_3 system exchange of deuterium at position 8 in 6-chloro-8-deuteriopurine and 8-deuterio-6-(methylthio)purine is very slow. Since we did not find spectroscopic evidence for the presence of 4 the conclusion seems justified that the formation of the σ-adduct 4 is the rate-determining step, and that the aromatization of 4 into the dianion of 2 by expulsion of the methylsulfide or chloride ion is fast. The instability of the σ-adduct 4 is in sharp contrast to the rather stable σ-adduct (4; X = R = H) observed as intermediate in the formation of 2 from purine itself. It was concluded that in the amination of purine not the formation of 4 (X = R = H) but the aromatization step is rate-determining.[3]

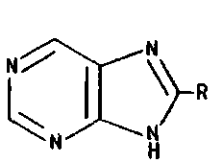
Table II *^{1}H NMR data of the mono- and dianions of 6- and 8-substituted purines and σ-adducts, in liquid ammonia containing potassium amide*

		H(2)	H(6)	H(8)	SCH_3
6-Cl-purine (1b)	mono anion	8.43	-	8.19	-
6-Cl-8-^{2}H-purine	mono anion	8.43	-	-	-
6-SCH_3-purine (1a)	mono anion	8.56	-	8.11	2.59
6-SCH_3-8-^{2}H-purine	mono anion	8.56	-	-	2.59
adenine (2)	dianion	7.64	-	7.34	-
		7.53	-	7.37	-
8-^{2}H-adenine	dianion	7.64	-	-	-
		7.53	-	-	-
8-Cl-purine (5b)	mono anion	8.55	8.65	-	-
	σ-adduct	6.87	5.58	-	-
8-SCH_3-purine (5a)	mono anion	8.49	8.57	-	2.67
	σ-adduct	6.95	5.73	-	2.36
6-^{2}H-8-SCH_3-purine	mono anion	8.49	-	-	2.67
	σ-adduct	6.95	-	-	2.36
6-t-C_4H_9-8-SCH_3-purine*	neutral($CDCl_3$)	8.72	-	-	2.78

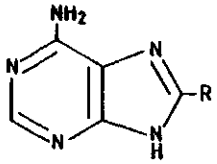
* the t-butyl group appears at 1.57 ppm

Amination of 8-methylthio- and 8-chloropurine

When 8-(methylthio)purine (5a) reacts with potassium amide in liquid ammonia at -33°C for 20 h 8-(methylthio)adenine (6a) is formed in 80% yield; 20% starting material can be recovered. No trace of 8-aminopurine (5c) can be detected.



5



6

a, R = SCH_3 ; b, R = Cl ; c, R = NH_2

The structure of 6a was proven by mass spectroscopy, UV spectroscopy[9] and conversion to adenine by Raney Nickel reduction as described for the removal of the methylthio group of 2-(methylthio)adenine[10]. Compound 5a has thus undergone a Chichibabin amination at position 6 (just as found for purine[3]) and not a substitution of the methylthio group at position 8. Reaction of 5a with ^{15}N-labelled potassium amide gave ^{15}N-labelled 8-(methylthio)adenine (6a*). Diazotization[7] of this product gave unlabelled 8-methylthiohypoxanthine (Table I), showing that the formation of 6a from 5a does not proceed via a ring-opening reaction. Measurement of the ^{1}H- and ^{13}C NMR spectra of a solution of 5a in liquid ammonia containing potassium amide showed first the presence of the anionic species, formed by deprotonation of 5a. Then a σ-adduct at position 6 is formed (4; X = H; R = SCH_3) as has also been observed for purine itself (4; X = R = H)[3]. That the addition indeed takes place at position 6 was unequivocally established by comparison with spectra of 6-deuterio-8-(methylthio)purine (Table II and III). The upfield shift of 2.84 ppm for H(6) and of 72.2 ppm for C(6) is in agreement with values reported in the literature.[3] This result shows that in the Chichibabin amination of 5a not the formation of the intermediate σ-adduct 4 (X = H, R = SCH_3) is rate determining (even if this intermediate is a dianion[3]), but the aromatization of the dianionic species 4 (X = H, R = SCH_3) into 6a. The reaction of 8-chloropurine (5b) with potassium amide in liquid ammonia is slower than the reaction of 5a and leads to a more complex reaction mixture. After 20 h 8-chloroadenine (6b) is formed in 10% yield only. The main product is adenine (2, 30%). Besides these two products about 50% of starting material can be recovered. No 5c could be detected in this reaction mixture. In order to establish whether a ring opening was involved in the formation of 2, the amination was carried out with ^{15}N-labelled potassium amide. We obtained labelled adenine (2*) which on diazotization[7] gave unlabelled hypoxanthine (3), thus showing that in the amination of 5b no ring-opening is involved either (Table

Table III ^{13}C *NMR data of the mono- and dianions of 6- and 8-substituted purines and σ-adducts in DMSO d-6* and liquid ammonia containing potassium amide.*

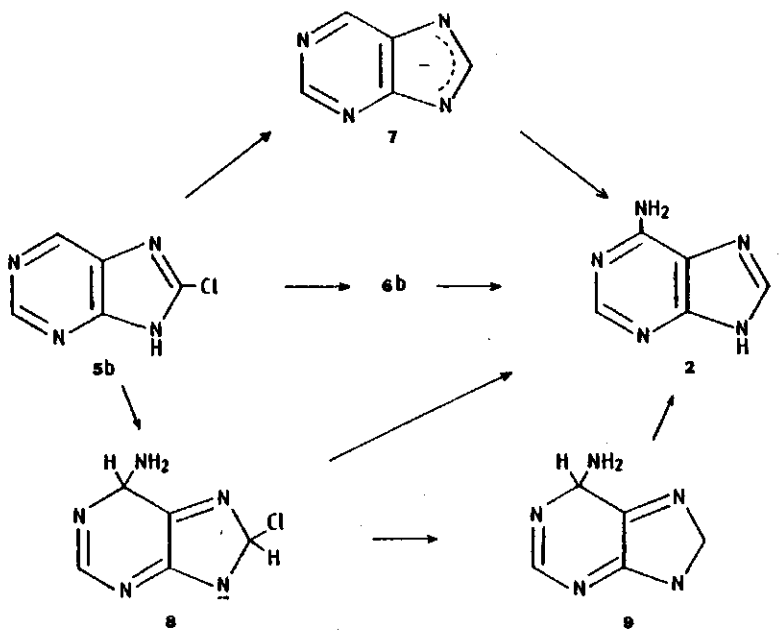
		C(2)	C(4)	C(5)	C(6)	C(8)	SCH_3
6-Cl-purine (1b)	neutral[19]	151.5	154.2	129.2	147.8	146.2	-
	anion	147.9	164.7	133.6	144.9	159.0	-
6-SCH_3-purine (1a)	neutral[19,20]	151.6	150.2	129.4	158.6	143.1	11.3
	anion	148.6	160.0	133.7	153.3	156.0	11.5
6-NH_2-purine (2)	neutral[19,20]	152.4	151.3	117.6	155.3	139.3	-
	dianion	151.6	156.2	124.3	167.7	146.4	-
		151.6	157.6	123.4	165.1	146.4	-
8-Cl-purine (5b)	neutral	149.5	158.2	133.2	140.9	150.1	-
	anion	149.5	163.6	137.8	142.0	157.5	-
	σ-adduct	153.4	148.3	118.8	66.5	132.6	-
8-SCH_3purine (5a)	neutral	151.1	158.0	132.0	141.6	156.7	13.4
	anion	148.5	165.2**	138.8	139.2	167.9**	13.8
	σ-adduct	153.6	149.5	121.0	67.0	138.6	18.7
6-t-C_4H_9-8-SCH_3--purine***	neutral ($CDCl_3$)	149.3	154.5**	133.1	166.3	153.6**	14.0

*In this solvent the neutral species are measured; the anions and σ-adducts are measured in the KNH_2/NH_3 system

**These assignments can also be interchanged

***The signals of the 6-tert-butyl group appear at 38.4 and 29.2 ppm. The coupling constant for C(2) (J_{C-H}= 202 Hz) shows that the tert-butyl group must be at position 6[3]

I). We did not investigate the formation of 6b with ^{15}N-labelled potassium amide due to the low yield of 6b, giving not enough material for diazotization. It seems justified to assume, based on the results of purine[3] and 5a, that 6b is formed through a Chichibabin amination of 5b without ring-opening.
Considering the formation of 2 from 5b several mechanisms can be advanced. The first pathway is dehalogenation at position 8 of 5b, followed by protonation at C(8), yielding the anionic purine 7, which is known[3] to react to 2 in the KNH_2/NH_3 system. Dehalogenation under these strong basic conditions is not uncommon.[11] However, since 7 is converted into adenine (2) for only 70% after 20 h and then unreacted purine can still be recovered,[3] we should expect a detectable amount of purine to be present in the reaction mixture obtained on amination of 5b, if 2 would be formed via 7. Despite many efforts this compound was not found, excluding to our opinion the intermediacy of 7. The second route i.e. amination of 5b into 6b, which then undergoes dehalogenation, could also be ruled out, as an independently prepared specimen of 6b was found to be unreactive with potassium amide after 20 h. From these results we have to conclude that 2 is formed through intermediate 8, which can either undergo a 1,4-tele-elimination into 2 or reacts first into 9 by a 1,2-dehydrochlorination followed by aromatization.



Although examples of tele-amination in heterocycles are known in the literature,[12] the occurence of a tele-amination in purines has never been described. Further evidence for this mechanism was obtained from the ^{1}H- and ^{13}C NMR spectra of a solution of 5b in liquid ammonia containing potassium amide. These spectra first showed the presence of the deprotonated species of 5b but soon a σ-adduct at position 6 (4; X = H, R = Cl) was observed (indicated by an upfield shift of 3.07 ppm for H(6) and of 75.5 ppm for C(6)). Since 8-chloropurine cannot be deuterated so easily as 8-(methylthio)purine the ^{1}H- and ^{13}C NMR spectra of the anion of 5b and the σ-adduct 4 (X = H, R = Cl) were assigned by comparison with the spectra of 8-(methylthio)purine (Table II and III). This result shows that although the chloro atom at position 8 is involved in the reaction, the first attack of the amide ion still takes place at position 6. It seems that the initial fast deprotonation of the imidazole ring in 5a as well as in 5b enhances the energy barrier for a direct nucleophilic attack on position 8 in this resonance-stabilized anion considerably, leading to a decreased reactivity for nucleophilic attack, particularly at that position.

We also studied the amination of 6-tert-butyl-8-(methylthio)purine[13] in which compound position 6 is now completely blocked against nucleophilic attack. It was found that even after 70 h 6-tertbutyl-8-(methylthio)purine is completely recovered, strongly suggesting that the reaction at position 8 can only take place via the intermediacy of an adduct at position 6. Also 8-aminopurine (5c) was found to be unreactive towards potassium amide in liquid ammonia even after 20 h. Apparently the formation of 6,8-diaminopurine (6c) is effectively hindered by the presence of the amino group at position 8, which just as the imidazole ring can easily be deprotonated. It is clear that in the dianionic species thus formed further nucleophilic substitutions are difficult.

Experimental Section

^{13}C- and ^{1}H NMR spectra were obtained with a Varian XL-100-15 spectrometer, equipped with a Varian 620/L16K computer. ^{1}H NMR spectra were recorded also on a Jeol C-60H spectrometer, equipped with a JES-VT-3 variable temperature controller. When measuring in DMSO d-6 internal TMS was used as standard. When measuring in liquid ammonia the sample temperature was ca. -50°C. For ^{13}C NMR spectra trimethylamine was used as internal standard. These spectra were converted to the TMS scale by adding 47.5 ppm. Typical ^{13}C spectral parameters were as follows: spectral width 5120 Hz, acquisition time 0.8 s, pulse delay 0-1.2 s, pulse

width 10 μs. For ^{1}H NMR spectra NH_3 was used as standard. The spectra were converted to the TMS scale by adding 0.95 ppm. Mass spectra and ^{15}N contents were determined on an AEI MS-902 mass spectrometer. IR spectra were obtained with a Perkin-Elmer 237 and an Hitachi EPI-G3 and UV spectra with a Beckman Acta CIII.

Preparation of starting materials

6-Chloropurine (1b),[4,6] 6-(methylthio)purine (1a),[14] 8-chloropurine (5b),[15] 8-aminopurine (5c)[5] and 8-chloroadenine (6b)[9] were prepared as described in the literature. 8-(Methylthio)purine (5a) was purchased from Aldrich and adenine (2) from Merck. ^{15}N-Labelled ammonia was prepared by reacting ^{15}N-labelled ammonium nitrate (from VEB Berlin-Chemie) with potassium hydroxide.

6-tert-Butyl-8-(methylthio)purine. 0.5 g of 8-(methylthio)purine, 60 mg of silver nitrate and 1,8 g of pivalic acid were dissolved in 25 ml of water.[16] With stirring at 60°C, 2.1 g of ammonium peroxydisulfate dissolved in 15 ml of water were added in 30 min followed by an additional 30 min stirring, while a pH of 1.5 was maintained. The solution was made slightly alkaline with aqueous sodium hydroxide and extracted continuously with chloroform. The extracts were dried ($MgSO_4$) the solvent was evaporated and the residue purified by column chromatography on silica gel using chloroform/ethylacetate 1:1 as eluent, followed by recrystallization from toluene/hexane.
Yield 12%, m.p. 192.5-193.5°C. Anal. Calcd for $C_{10}H_{14}N_4S$, C, 54.02; H, 6.35, Found C, 53.68; H, 6.84. The structure was proven by ^{1}H- and ^{13}C NMR spectroscopy (see Table II and III).

Preparation of 6- and 8-deuteriopurines. 6-Chloro-8-deuteriopurine, 8-deuterio-6-(methylthio)purine and 8-deuterioadenine were obtained by refluxing 6-chloropurine (1b), 6-(methylthio)purine (1a) and adenine (2) respectively in deuterium oxide.[17] The position of deuteration was proven by ^{1}H NMR spectroscopy.[18]

6-Deuterio-8-(methylthio)purine was prepared by heating 8-(methylthio)purine (5a) for 4 h at 140°C in deuteriumoxide. This method gives in a low yield (10%) 8-(methylthio)purine containing about 80% deuterium. Refluxing of 5a for 75 h with 10% palladium on charcoal as catalyst at 100°C leads to only 20% of deuterium incorporation. The position of deuteration was established by ^{13}C NMR spectroscopy, because the assignment of the proton signals was unknown. For NMR measurements the deuterium labelled compounds were diluted to about 50% deuterium content.

Amination procedure. The amination procedure was carried out in exactly the same manner as described in a previous paper[3].

Acknowledgement

This investigation was carried out under the auspices of the Netherlands Foundations for Chemical Research (SON) and with financial aid from the Netherlands Organisation for Advancement of Pure Research (ZWO). We are indebted to Dr. G.B.Brown (The Sloan-Kettering Institute for Cancer Research, Walker Laboratory, Rye, New York) for a gift of 6-mercaptopurine, Drs.C.A.Landheer and Mr.W.P. Combé for the mass spectroscopic data, Mr.A.Koudijs for help with the synthesis of 6-tert-butyl-8-(methylthio)purine and Mr.H.Jongejan for carrying out the microanalysis.

References and Notes

(1) Part LXXIX on pyrimidines from this laboratory. For part LXXVIII see J.Breuker and H.C.van der Plas, J. Org. Chem., in press.
(2) For a review: J.H.Lister in the Chemistry of Heterocyclic compounds. Fused Pyrimidines, Part II, D.J.Brown, Ed., Wiley, New York, 1977.
(3) N.J.Kos, H.C.van der Plas and A.van Veldhuizen, J. Org. Chem., 44, 3140 (1979).
(4) G.B.Barlin, J. Chem. Soc. B 954 (1967).
(5) A.Albert and D.J.Brown, J. Chem. Soc., 2060 (1954).
(6) A.Bendich, P.J.Russel Jr and J.J.Fox, J. Am. Chem. Soc., 76, 6073 (1954).
(7) M.Krüger, Z. Physiol. Chem., 18, 444 (1886).
(8) The term S_N(ANRORC) mechanism refers to a reaction, which involves Addition of the Nucleophile, Ring Opening and Ring Closure. For a very recent review on this subject see H.C.van der Plas, Accounts of Chem. Res., 11, 462 (1978).
(9) R.K.Robins, J. Am. Chem. Soc., 80, 6671 (1958).
(10) E.C.Taylor, O.Vogel and C.C.Cheng, J. Am. Chem. Soc., 81, 2442 (1959).
(11) See for example: A.Rykowski and H.C.van der Plas, J. Org. Chem., 45, 881 (1980).
(12) See for example: H.C.van der Plas, M.Wozniak and A.van Veldhuizen, Recl. Trav. Chim. Pays-Bas, 96, 151 (1977).
(13) This compound provides in principle an easy access to 6-t-butylpurine, prepared previously by an eight step procedure (G.K.Helmkamp and N.S.Kondo, Biochim. et Biophys. Acta, 157, 242 (1968).

(14) G.B.Elion, E.Burgi and G.H.Hitchings, J. Am. Chem. Soc., 74, 411 (1952).
(15) A.G.Beaman and R.K.Robins, J. Appl. Chem., 12, 432 (1962).
(16) The same procedure was described for the t-butylation of pyrimidines.See H.C.van der Plas and A.Koudijs, Recl. Trav. Chim. Pays-Bas, 97, 159 (1978).
(17) R.J.Pugmire, D.M.Grant, R.K.Robins and G.W.Rhodes, J. Am. Chem. Soc., 87, 2225 (1965).
(18) F.J.Keck Jr, R.A.Simpson and J.L.Wong, J. Org. Chem., 43, 2587 (1978).
(19) M.C.Thorpe, W.C.Coburn Jr and J.A.Montgomery, J. Magn. Reson,, 15, 98 (1974).
(20) M.T.Chenon, R.J.Pugmire, D.M.Grant, R.P.Panzica and L.B.Townsend, J. Am. Chem. Soc., 97, 4636 (1975).

4 ON THE OCCURENCE OF THE S_N(ANRORC) MECHANISM IN THE AMINATION OF 2-SUBSTITUTED PURINES WITH POTASSIUM AMIDE IN LIQUID AMMONIA

Nico J. Kos and Henk C. van der Plas

The reactions of 2-chloro-, 2-fluoro- and 2-(methylthio)purine with potassium amide in liquid ammonia lead to the formation of 2-aminopurine. When these reactions are carried out with ^{15}N-labeled potassium amide, ring labeled 2-aminopurine is found. This demonstrates that a ring opening occurs during the amination. Formation of an anionic σ-adduct at position 6 is proven by low temperature NMR spectroscopy, and evidence is obtained for the formation of an open chain intermediate, although this intermediate could not be isolated in pure state. Reaction of the open-chain intermediate with hydriodic acid gives the thus far unknown 2-iodopurine. 2-Chloro-6-phenylpurine also reacts via ring-opening into 2-amino-6-phenylpurine. However, 2-chloro-6-methyl- and 2-chloro-6,8-di-tert-butylpurine are found to be unreactive.

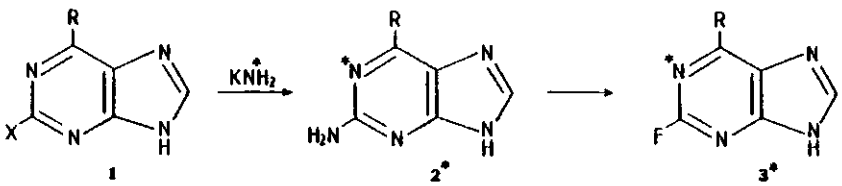
Introduction

We have recently found that purine and its derivatives containing a leaving group at position 6 or 8 easily undergo amination with potassium amide in liquid ammonia.[2,3] From purine and the 6-substituted purines adenine is obtained, being formed by an addition-elimination reaction at position 6.[2,3] Interestingly 8-chloro- and 8-(methylthio)purine do not undergo amination at position 8, but also at position 6 giving 8-chloroadenine (together with adenine) and 8-(methylthio)adenine respectively. These reactions have been explained by an initial addition of the amide ion at position 6 leading to a σ-adduct, which undergoes either aromatization into 8-chloro-or 8-(methylthio)-adenine or an 1,4-tele-elimination into adenine.[3] In principle 2-substituted purines are appropriate compounds to give addition at position 6.[2,3] Therefore, these compounds could give 2-aminopurines, via a ring opening (S_N(ANRORC)-mechanism).[4] In order to prove it several 2-substituted purines were synthesized and their behaviour towards potassium amide was studied.

Results and Discussion

2-Chloro, 2-fluoro- and 2-(methylthio)purine

When 2-chloro-(1a) or 2-fluoropurine (1b) reacts with potassium amide in liquid ammonia for 20 h and the reaction mixture is worked up as usually[2] 2-aminopurine (2, R = H) is formed in good yield (80% and 60% respectively). 2-(Methylthio)purine (1c) gives a similar reaction: after 70 h 2-aminopurine (2, R = H) is obtained in 90% yield. In contrast 1a and 1c are expected to be unreactive with aqueous ammonia to give 2 (R = H).[5,6] When the reactions of 1a-1c are followed by TLC, it became evident, that in the reactions of all three purines (1a - 1c), a compound is formed, which quickly converts into 2-aminopurine (2, R = H) during work up. This precursor of 2 (R = H) is the same in all three reactions. This intermediate is not sufficiently stable to be isolated in pure state, since conversion into 2 (R = H) takes place during isolation. Therefore only an IR spectrum of the mixture of this intermediate and 2-aminopurine could be obtained, it showed i.a. an absorption at 2160 cm^{-1}, being characteristic for the presence of a conjugated N-CN group. This result indicates that this intermediate is formed by an opening of the pyrimidine nucleus, which is then followed by cyclisation into 2 (R = H). To prove this ring opening the amination of 1a - 1c was carried out with ^{15}N-labeled potassium amide. We found that in the labeled 2-aminopurine (2*, R = H) the label was present exclusively in the ring nitrogens; no trace of ^{15}N was found in the nitrogen of the amino group. This was proven by conversion of 2* (R = H) into 2-fluoropurine (3*, R = H) by diazotization in fluoroboric acid[7] and by determining the ^{15}N content in 2* (R = H) and 3*(R = H) using mass spectrometry (Table I).



1 2* 3*

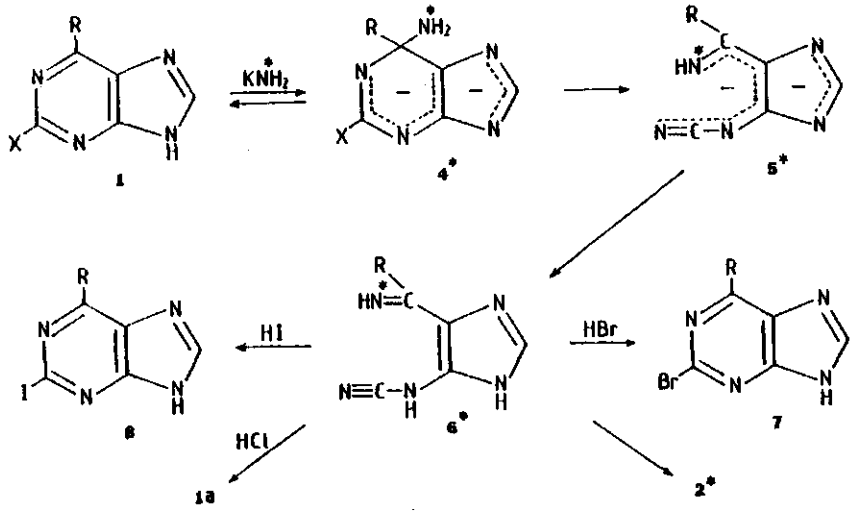
a. R = H , X = Cl
b. R = H , X = F
c. R = H , X = SCH_3
d. R = CH_3 , X = Cl
e. R = C_6H_5 , X = Cl

Table I ^{15}N *contents (percentages) of the products obtained in the reaction of the starting compounds with* ^{15}N*-labeled potassium amide*

products / starting compound	2-NH_2-(6-R-)purine	2-F-(6-R-)purine
2-Cl-purine (1a)	6.2 (5.7) (R=H)	6.4 (5.6) (R=H)
2-F-purine (1b)	6.0 (5.7) (R=H)	5.8 (5.7) (R=H)
2-SCH_3-purine (1c)	4.1 (5.4) (R=H)	4.3 (5.8) (R=H)
2-Cl-6-C_6H_5-purine (1e)	6.1 (5.0) (R=C_6H_5)	5.6 (4.6) (R=C_6H_5)

The numbers in brackets refer to duplicate experiments; the accuracy of the measurements is ± 0.2 %.

These results show that an S_N(ANRORC)-mechanism[4] operates in the amination of 1a - 1c, involving initial addition of the amide ion at position 6 in the deprotonated purine, giving a dianionic σ-adduct (4, R = H), that can undergo a ring opening into intermediate 5 (R = H).[8] This intermediate 5 (R = H) is stable in the potassium amide/liquid ammonia solution. Addition of ammonium sulfate (necessary to neutralize the potassium amide), gives the neutral species 6 (R = H) in which cyclisation of the side chain occurs by an attack of the imino group on the carbon atom of the cyanamino group.
Addition of the amide ion at position 6 in the purine anion is in full agreement with earlier observations.[2,3] Additional evidence for addition at position 6 was obtained by ^1H NMR spectroscopy. A solution of 1a - 1c in liquid ammonia containing potassium amide (0.15 - 0.5 mmol of 1a - 1c in 1 ml of liquid ammonia containing 4-10 equivalents of potassium amide) showed first the formation of the anion of 1a - 1c, then the formation of a (very short lived) σ-adduct as indicated by upfield shifts of 2.4 - 3.0 ppm for one of the hydrogens[2,3] (Table II). Comparison with the spectra of solutions of 2-chloro-8-deuterio- and 8-deuterio-2-(methylthio)purine in KNH_2/NH_3 proves that the σ-adduct formation takes place at position 6. In the solution of 1c after some



time a signal appeared at 1.78 ppm assigned to SCH_3^-, formed during the conversion of 4 (R = H, X = SCH_3) into 5 (R = H), The observation that the intermediates obtained from 1a - 1c are identical on TLC, indicates that also in case of 1a and 1b the formation of 5 occurs with loss of Cl^- or F^- respectively. It is interesting to note that 2-fluoropurine (1b) exclusively reacts via a ring opening mechanism into 2-aminopurine (2, R = H), since there are several amino-defluorinations reported in the literature in which the S_N(AE) mechanism is a more important pathway than the S_N(ANRORC)-mechanism.[4,9] The fact that 2-chloropurine reacts via an S_N(ANRORC)-mechanism into 2-aminopurine gives support to the suggestion that the reaction of 2-chloro-7-methyladenine with sodium hydroxide leading to the formation of 2-amino-6-hydroxy-7-methylpurine also proceeds via this mechanism, although an intermediate could never be identified.[10,11]

Reaction of 6 (R = H) with conc. hydrochloric acid yields 2-chloropurine[8] (1a, 87%) while 2-bromopurine (7, R = H, 55%) and 2-iodopurine (8, R = H, 50%) are formed with hydrobromic and hydriodic acid respectively. Specially the preparation of 2-iodopurine is of interest[12] as this compound was unknown till now. Reaction of 6 (R = H) with hydrofluoric acid gave no 2-fluoropurine, but only 2-aminopurine. This can be explained by the lower nucleophilicity of the fluoride ion in comparison with the other halogen ions. Compound 6 obtained in a reac-

Table II *^{1}H-NMR data (δ values) of 2-substituted purines in liquid ammonia containing potassium amide*

		H-6	H-8	SCH_3
2-Cl-purine (1a)	anion	8.61	8.11	-
	adduct	5.62	6.85	-
2-Cl-8-D-purine	anion	8.61	-	-
	adduct	5.62	-	-
2-F-purine (1b)	anion	8.54	8.08	-
	adduct	6.08	6.73	-
2-SCH_3-purine (1c)	anion	8.66	8.03	2.57
	adduct	6.23	6.98	2.35
2-SCH_3-8-D-purine	anion	8.66	-	2.57
	adduct	6.23	-	2.35
SCH_3^-		-	-	1.78
2-Cl-6-CH_3-purine (1d)	dianion*		6.97	

* A CH_2^- signal appeared as two doublets (3.06 and 3.21 ppm, J = 3 Hz).

tion with ^{15}N-labeled potassium amide gave after treatment with hydrochloric acid 2-chloropurine (1a) in which no label was found.

2-Aminopurine

The fact that 2-methylpurine reacts with potassium amide to give 2-methyladenine,[1] induced us to react 2-aminopurine (2, R = H) with potassium amide in liquid ammonia with the aim to synthesize 2,6-diaminopurine. Even after 20 h we found no product; 2-aminopurine could be recovered quantitatively. This indicates that in contrast to 2-methylpurine both the NH_2-group as well as the imidazole ring in 2-aminopurine are deprotonated yielding a dianion i.e. a species in which position 6 is effectively deactivated for nucleophilic attack.

2-Chloro-6-substituted purines

The influence of a substituent at position 6 in 2-chloropurines on the mechanism of the amino-dechlorination was also studied. If addition of the amide ion at position 6 is retarded or even prohibited by the presence of the substituent, the replacement of the chloro atom by the amino group might occur partly or completely via the alternative S_N(AE)-mechanism.

Reaction of 2-chloro-6-methylpurine (1d) with potassium amide in liquid ammonia gave after 70 h 6-methylpurine (10%) besides starting material (90%). We explain the absence of a 2-amino compound by the formation of a dianion through proton abstraction of both the NH in the imidazole ring and the methyl group.[2] This dianion is highly deactivated towards nucleophilic attack. An ^{1}H NMR spectrum of 1d dissolved in liquid ammonia containing potassium amide indeed showed the presence of a dianion as illustrated i.a. by the appearance of the CH_2^- signal as two doublets[2] (Table II). The formation of 6-methylpurine is due to dehalogenation, not unprecedented in a strongly basic medium.[13] We also studied the amination of 2-chloro-6-phenylpurine (1e). This coumpound can only give a monoanion and therefore can be expected to react "easier" than 1a with potassium amide. After 70 h we indeed isolated 2-amino-6-phenylpurine (2, R = C_6H_5) in a yield of 80% besides 20% of starting material. When the reaction was carried out with ^{15}N-labeled potassium amide we obtained labeled 2-amino-6-phenylpurine (2*, R = C_6H_5), which by diazotization[7] yielded 2-fluoro-6-phenylpurine (3*, R = C_6H_5), containing all the ^{15}N-label (Table I). This shows that the formation of 2 (R = C_6H_5) from 1e has proceeded via an S_N(ANRORC)-mechanism (> 90%). Thus despite the presence of a "bulky" group at position 6, the addition takes place at that position. Addition of an amide ion to a position being substituted by a phenylgroup is not unprecedented.[9,14] Finally we tested whether the more voluminous tert-butyl group at position 6 could change the mechanism of the amination from S_N(ANRORC) to S_N(AE). 2-Chloro-6,8-di-tert-butylpurine was synthesized, but found to be unreactive, even after 70 h. The same result was reported with 6,8-di-tert-butylpurine.[2] Summarizing all the results obtained thus far we have to conclude that a direct attack of the amide ion at position 2 does not take place even when a leaving group is present at that position. Since we have already shown that also position 8 is completely unreactive towards potassium amide,[3] the conclusion seems justified that a purine derivative that gives a monoanion by deprotonation of the imidazole ring under influence of the amide ion, only reacts with the amide ion at position 6. These experimental results are in agreement with data, given in the literature,

showing that in anionic purines position 6 is more reactive than position 2 or 8 (See ref. 12).

Experimental Section

^{13}C and ^{1}H NMR spectra were obtained with a Varian XL-100-15 spectrometer, equipped with a Varian 620/L16K computer. ^{1}H NMR spectra were recorded also on a Jeol C-60H spectrometer, equipped with a JES-VT-3 variable temperature controller. When measuring in DMSO d-6 internal TMS was used as standard. When measuring in liquid ammonia the sample temperature was ca. $-50^{o}C$ and NH_3 was used as standard. The spectra were converted to the TMS scale by adding 0.95 ppm. Mass spectra and ^{15}N contents were determined on an AEI MS-902 mass spectrometer. IR spectra were obtained with a Perkin-Elmer 237 and an Hitachi EPI-G3 and UV spectra with a Beckman Acta CIII.

Preparation of Starting Materials

2-Aminopurine (2, R = H) was purchased from Sigma. 2-Chloropurine (1a)[15,16] and 2-fluoropurine (1b)[7] were prepared as described in the literature. 2-(Methylthio)purine (1c) and 2-chloro-6-methylpurine (1d) were prepared by reacting 2-methylthio-4,5-diaminopyrimidine[6] and 2-chloro-4,5-diamino-6-methylpyrimidine[17] respectively with diethoxymethylacetate.[15,18]

2-Chloro-6-phenylpurine (1e). 2-Chloro-4,5-diamino-6-phenylpyrimidine[16] (0.35 g) was refluxed in 10 ml of diethoxymethylacetate[18] for 3 h. The solvent was evaporated in vacuo and the residue recrystallized from ethanol; yield 60%, mp 273-274°C. Anal. Calcd. for $C_{11}H_7ClN_4$: C, 57.28; H, 3.06. Found: C, 57.23; H, 3.06.

2-Chloro-6,8-di-tert-butylpurine. 2-Chloropurine (650 mg), 150 mg of silver nitrate and 5.5 g of pivalic acid were dissolved in water (25 ml).[19] With stirring 15 g of ammoniumperoxydisulfate dissolved in 30 ml of water were added at 75°C in 30 min, followed by an additional 30 min stirring. The solution was made alkaline with aqueous sodium hydroxide and extracted with chloroform. The extracts were dried ($MgSO_4$), the solvent was evaporated and the residue recrystallized from hexane or aqueous methanol followed by sublimation at 0.1 mm, yield 60%, mp 179-180°C. Anal. Calcd. for $C_{13}H_{19}ClN_4$: C, 58.53; H, 7.18. Found: C, 58.64; H, 7.17.

2-Iodopurine. The crude product obtained by reaction of 2-chloropurine or 2-(methylthio)purine with potassium amide (reaction time 25 and 70 h respectively), subsequent treatment with ammonium sulfate and evaporation of the ammonia was immediately treated with 47% hydriodic acid for 1h. The solution was neutralized with aqueous sodium hydroxide and continuously extracted with ethylacetate. Two recrystallizations from water yielded pure 2-iodopurine (50%), mp 233-236°C. Anal. Calcd. for $C_5H_3JN_4$: C, 24.41; H, 1.23. Found: C, 24.38; H, 1.08. The crude product mentioned above yielded 2-chloropurine (87%) with 36% hydrochloric acid and 2-bromopurine (55%) when 47% hydrobromic acid was used. The identity of the 2-chloro and 2-bromopurine was established by UV and mass spectrometry.

Preparation of 8-deuteriopurines. 2-Chloro-8-deuteriopurine and 8deuterio-2-(methylthio)purine were obtained by refluxing 2-chloropurine and 2-(methylthio)purine respectively for 4 h in deuterium oxide.[2,3,20] The position of deuteration was proven by NMR spectroscopy.[21,22] For NMR measurements the deuterium labeled compounds were diluted to about 50% deuterium content.

Amination Procedure. The amination reactions were carried out in exactly the same manner as described in a previous paper.[2] The amination of the compounds 1a-1d gave known products. However from 1e 2-amino-6-phenylpurine (2, R = C_6H_5) mp 257-259°C was obtained. The structure of this product was proven by ^1H NMR: ($CDCl_3/CD_3OD$): δ 7.98 (s, 1H); 7.49 and 8.36 (m, 5H); ^{13}C NMR (Me_2SO-d_6): C-2, 160.3; C-4, 155.8; C-5, 123.7; C-6, 153.0; C-8, 140.9 (J = 210 Hz); C_6H_5, 128.3, 129.1, 130.4 and 136.3 ppm. UV, $\lambda_{max}(CH_3OH)$ 331 nm; Anal. Calcd. for $C_{11}H_9N_5$: C, 62.54; H, 4.30. Found: C, 62.28; H, 4.24.

Acknowledgement

This investigation was carried out under the auspices of the Netherlands Foundations for Chemical Research (SON) and with financial aid from the Netherlands Organisation for Advancement of Pure Research (ZWO). We are indebted to Drs. C. A. Landheer and Mr. W. P. Combé for the mass spectroscopic data, Mr. A. van Veldhuizen for measuring the NMR spectra, Mr. H. Jongejan for carrying out the microanalyses and Mr. A. Koudijs for the synthesis of 2-chloro-6,8-di-tert-butylpurine.

References

(1) Part XXVII on the S_N(ANRORC) mechanism; For Part XXVI see A. Rykowski and H.C.van der Plas, J. Org. Chem., in press. Pyrimidines, Part LXXXI. For Part LXXX see R.E.van der Stoel, H.C.van der Plas, H.Jongejan and L.Hoeve, Recl. Trav. Chim. Pays-Bas, in press.

(2) N.J.Kos, H.C.van der Plas and A.van Veldhuizen, J. Org. Chem., 1979, 44, 3140.

(3) N.J.Kos, H.C.van der Plas and A.van Veldhuizen, Recl. Trav. Chim. Pays-Bas, in press.

(4) The term S_NANRORC mechanism refers to a reaction involving an addition of the nucleophile, ring opening, and ring closure. For a review, see: H.C.van der Plas, Acc. Chem. Res. 1978, 11, 462.

(5) H. Balweg, Justus Liebigs Ann. Chem., 1961, 649, 114.

(6) A.Albert and D.J.Brown, J. Chem. Soc., 1954, 2060.

(7) J.A.Montgomery, K.Hewson, Am. Chem. Soc., 1960, 82, 463.

(8) A.P.Kroon and H.C.van der Plas, Recl. Trav. Chim. Pays-Bas, 1973, 92, 1020.

(9) A.P.Kroon and H.C.van der Plas, Recl. Trav. Chim. Pays-Bas, 1974, 93, 227.

(10) E.Fischer, Ber., 1898, 31, 542.

(11) E.Shaw, J. Org. Chem., 1962, 27, 883.

(12) It is suggested that 2-iodopurine cannot be prepared in the usual way through reaction of 2-chloropurine with hydriodic acid. For a review, see: J.H.Lister, Chem. Heterocycl. Compd., 1971, 143, 144.

(13) See for example: A.Rykowski and H.C.van der Plas, J. Org. Chem., 1980, 45, 881.

(14) Gy Simig, H.C.van der Plas and C.A.Landheer, Recl. Trav. Chim. Pays-Bas, 1976, 95, 113. A.Nagel and H.C.van der Plas, Heterocycles, 1977, 7, 205.

(15) J.A.Montgomery, C.Temple Jr., J. Org. Chem., 1960, 25, 395.

(16) A.Nagel, H.C.van der Plas and A.van Veldhuizen, Recl. Trav. Chim. Pays-Bas, 1975, 94, 45.

(17) S.Gabriel and J.Colman, Ber., 1901, 34, 1234.

(18) J.W.Scheeren and W.Stevens, Recl. Trav. Chim. Pays-Bas, 1966, 85, 793.

(19) See for the same procedure: H.C.van der Plas and A.Koudijs, Recl. Trav. Chim. Pays-Bas, 1978, 97, 169.

(20) R.J.Pugmire, D.M.Grant, R.K.Robins and G.W.Rhodes, J. Am. Chem. Soc., 1965, 87, 2225.

(21) G.B.Barlin and A.C.Young, J. Chem. Soc. B, 1971, 821.

(22) D.J.Brown and P.W.Ford, J. Chem. Soc. C, 1969, 2620.

5 DEAMINATION OF 6-AMINO- AND 6-(ALKYLAMINO)-9-ALKYLPURINES AND DEMETHYLATION OF METHYLTHIOPURINES BY SODIUM IN LIQUID AMMONIA

Nico J. Kos and Henk C.van der Plas

6-Amino- and 6-(alkylamino) purines, having an alkyl substituent at position 9, deaminate when treated with sodium in liquid ammonia. The reaction involves a 6-amino-1,6-dihydro-9-alkylpurine as intermediate, as proved by ^{1}H NMR spectroscopy. Sodium in liquid ammonia can also be used to demethylate 6- and 8-methylthiopurines.

Introduction

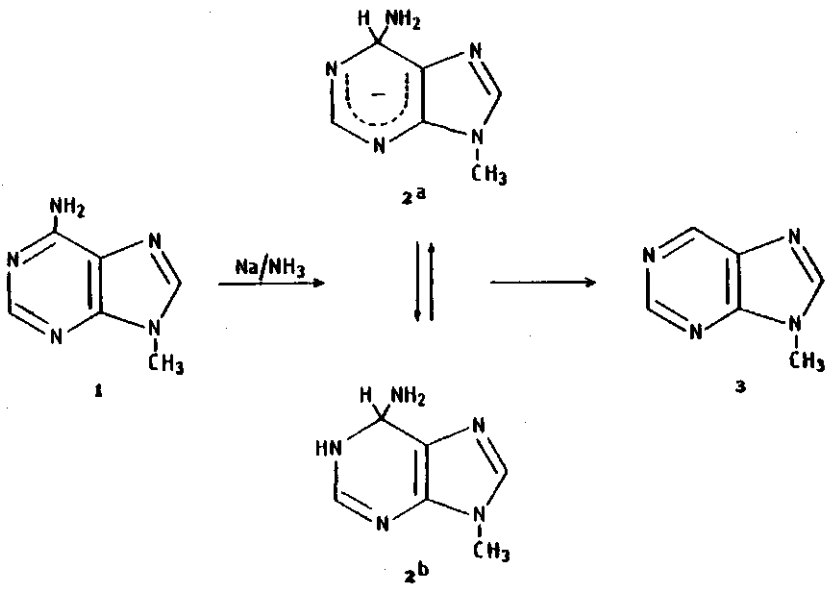
Several reagents have been usefully applied for the reduction of a C=N bond in purines. With sodium borohydride reduction can occur at different positions.[3-6] When this reagent is used for the reduction of 7- or 9-alkylchloropurines it has been reported to occur without loss of the chloro atom.[7] Treatment of thiopurines with Raney nickel catalyst gives besides desulfurization also reduction of the purine ring, yielding dihydropurines. Electrochemical methods[3,8] and hydrogenation on metal catalysts have also been applied, but the second method frequently leads to ring opening.[9] Sodium in liquid ammonia as reducing agent in purine chemistry has been reported for removing benzyl groups from N- and S-benzylpurines.[3]

Results and Discussion

We found that on treatment of 6-amino-9-methylpurine (1) with sodium in liquid ammonia and subsequent addition of ammonium sulphate (to quench the reaction mixture) 9-methylpurine (3) was obtained in a yield of about 45%.(Table II, Scheme I). This yield did not considerably improve by increasing the reaction time, by addition of the proton donor ethanol or by using tetrahydrofuran or diethylether as cosolvent, although with diethylether less by-products are formed. Replacement of sodium by lithium or potassium gave inferior results. This simple method to replace the amino group at position 6 in 9-alkylpurines by a hydrogen atom is synthetically useful, since up to now deamination could only

be effected by diazotization.[10]

We assume that the first step in the conversion of 1 into 3 involves reduction of the N(1) - C(6) bond to give as intermediate the anion of 1,6-dihydropurine (2a) or its conjugate acid (2b). Loss of the amide ion from 2a or ammonia from 2b yields 3 (Scheme I).[11]



We found ^{1}H NMR spectroscopic evidence for the intermediary existence of 2, since the spectrum of the reaction mixture, obtained by adding sodium to a solution of 1 in liquid ammonia in such an amount that the blue color just disappears (thus without quenching of this solution with ammonium sulphate) displayed besides signals of the starting material the presence of a triplet at 5.62 ppm (Table I), being characteristic for the H(6) in intermediate 2.

This chemical shift is nearly the same as found previously for the H(6) singlet in the σ-adduct 4 (X=H; 5.75 ppm) formed when purine is dissolved in liquid ammonia containing an excess of potassium amide.[12]

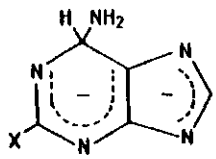
That the H(6) signal at 5,62 ppm in 2 is present as a triplet, while in the σ-adduct 4 (X=H) this signal appears as a singlet is due to the fact that during the formation of 2 only a small amount of sodium amide is formed, while the formation of 4 (X=H) occurs in the presence of an excess of potassium amide, leading to a rapid exchange of the hydrogen of the amino group.

Table I. ^1H *NMR data (δ values) in liquid ammonia*

	H-2	H-6	H-8	CH_3
6-NH_2-9-CH_3-purine (1)	7.72	-	7.68	3.67
6-NH_2-8-D-9-CH_3-purine	7.72	-	-	3.67
1,6-dihydro-6-NH_2-9-CH_3-purine (2)	7.08	5.62[a]	7.01	3.43
1,6-dihydro-6-NH_2-8-D-9-CH_3-purine	7.08	5.62[a]	-	3.43
1,6-dihydro-6-NH_2-purinide (4, X=H)[b]	6.95	5.75[c]	6.83	-

[a] triplet (J= 7 Hz)
[b] obtained on dissolving purine in KNH_2/NH_3[12]
[c] singlet

In 2 this rapid exchange does not take place and a coupling of H(6) with the protons of the amino group is now observed.[12]



4

Also the upfield shifts of the signals for H(2) and H(8)are in agreement with the formation of intermediate 2.[12] That the signal of 7.08 ppm is correctly assigned to H(2) and that of 7.01 ppm to H(8) was proved by synthesizing 6-amino-8-deuterio-9-methylpurine and comparing the ^1H NMR spectrum of the deuterium intermediate obtained after treatment with sodium in liquid ammonia with that of the protium derivative 2. These results show clearly that the deamination reaction proceeds *via* a reduction of the N(1) - C(6) bond followed by elimination. The elimination reaction takes place on quenching with ammonium sulphate, which explains why 3 can be isolated in reasonable yield, although it is not stable in liquid ammonia, containing potassium amide.
The successful deamination of 1 into 3 induced us to investigate the scope of this reaction. We attempted to deaminate adenine into purine, but found that adenine does not react. Apparently the presence of an alkyl group at position 9 is necessary for a successful deamination.[13] However, when we reacted 6-amino-9-[2'-tetrahydropyranyl] purine with sodium in liquid ammonia, 9-[2'-tetrahydropyranyl]purine was obtained. Acid hydrolysis gave purine.[14]

We also found that deamination of adenosine into nebularine does not occur, but that the reductive removal of the amino group from 2',3'-O-isopropylidene adenosine into 2',3'-O-isopropylidene nebularine easily takes place. Reductive removal is not confined to the amino group, but substituted amino groups too are found to be easily replaced by hydrogen; reduction of 6-methylamino-9-methylpurine and 6-dimethylamino-9-methylpurine gave 9-methylpurine. The direct replacement of a substituted amino group by hydrogen has not been described before, since diazotization[10] cannot be applied with substituted amino groups.

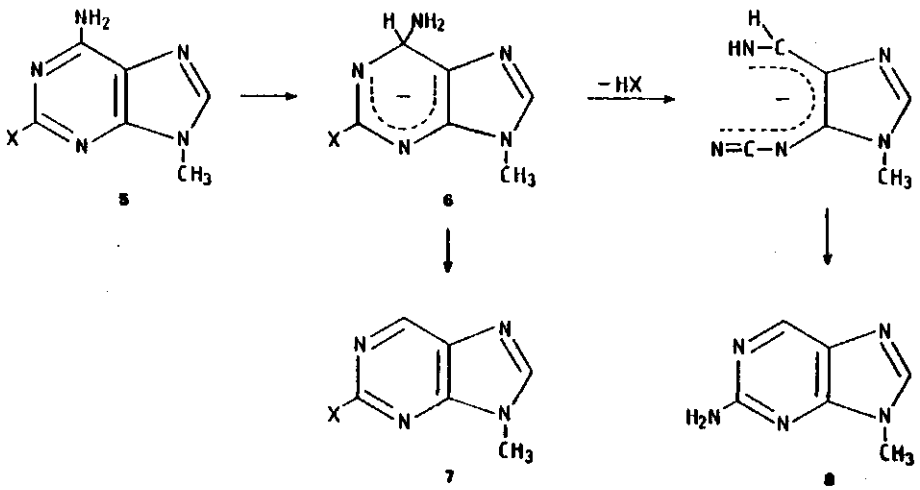
Table II. Reaction procedures and yields for the reduction of purines

starting compound	product	reaction time (min)	diethyl ether added	Yield %[a]
6-NH_2-9-CH_3-purine (1)	9-CH_3-purine	30	-	45
		60	-	46
		120	-	43
		30	+	40
6-NH_2-9-[2'-tetrahydropyranyl] purine	9-[2'-tetrahydropyranyl] purine	30	+	40
2',3'-O-isopropylidene adenosine	2',3'-O-isopropylidene nebularine	30	+	63[b]
6-$NHCH_3$-9-CH_3-purine	9-CH_3-purine	30	-	42
		60	-	40
6-$N(CH_3)_2$-9-CH_3-purine	9-CH_3-purine	15	+	53
		30	+	55
2-Cl-6-NH_2-9-CH_3-purine	6-NH_2-9-CH_3-purine[c]	30	+	47
2-OCH_3-6-NH_2-9-CH_3-purine	2-OCH_3-9-CH_3-purine	30	+	60[d]
2,6-di-NH_2-9-CH_3-purine	2-NH_2-9-CH_3-purine	30	+	60
6-SCH_3-purine	6-SH-purine	10	-	40[d]
8-SCH_3-purine	8-SH-purine	5	-	100
8-SCH_3-9-CH_3-purine	8-SH-9-CH_3-purine	5	-	100

[a] Due to the small scale of the preparation, the yields were determined in duplicate by NMR spectroscopy of the reaction mixture.
[b] On a larger scale (250 mg) the yield was only 25%.
[c] Besides some 9-CH_3-purine.
[d] For this compound the yield could not be determined by NMR spectroscopy due to the formation of byproducts. Therefore we have given the isolated yield.

From our amination studies with purines[15], containing a leaving group at position 2, it has unequivocally been established that these compounds undergo an initial adduct formation at position 6, yielding a 6-amino-1,6-dihydropurine (4, X=Cl, F, SCH_3). This adduct undergoes a ring opening reaction, leading to an open-chain intermediate that gives ring closure into 2-aminopurine (S_N(ANRORC) mechanism).

Since in this paper it has been shown that a 6-amino-1,6-dihydropurine can also be obtained by reduction of a 6-aminopurine, we became interested in the behaviour of 6-aminopurines, containing at position 2 a leaving group *i.e.* 5. It is possible that on reduction of compound 5 intermediate 6 is obtained which then may undergo a ring opening - ring closure sequence affording 2-amino-9-methylpurine 8 (5 → 6 → 8; Scheme III).



On treatment of 6-amino-2-chloro-9-methylpurine (5, X=Cl) with sodium in liquid ammonia, it was observed that the halogen atom was very quickly lost, giving 6-amino-9-methylpurine, which yielded on further reduction 9-methylpurine. Reduction of 6-amino-2-methoxy-9-methylpurine (5, X=OCH_3) gave 2-methoxy-9-methylpurine (7, X=OCH_3) as sole product. No trace of 2-amino-9-methylpurine (8) was found. The conclusion is evident: no ring opening has occurred, but only elimination of amide ion (5 → 6 → 7; X=OCH_3). It is interesting that reduction of 2,6-diamino-9-methylpurine (5, X=NH_2) selectively removes the amino group from position 6, yielding 2-amino-9-methylpurine (7, X=NH_2 or 8). This product is very likely formed *via* elimination of ammonia from N(1) - C(6)

[route (5 → 6 → 7; X=NH_2)] .

All the results mentioned before clearly indicate that it is the amino group at position 6 and not at position 2, which can be reductively removed. This result can be understood in the light of the fact that the introductory step in the reductive deamination is the addition of the nucleophilic hydride ion and that the purine ring favours addition at position 6.

Attempts to perform reductive removal of methoxy and methylthio groups[16] from position 6 in 9-methylpurines failed ; 6-methoxy-9-methylpurine and 6-methylthio-9-methylpurine could not be converted into 9-methylpurine.

However, 8-methylthio-9-methylpurine was found to undergo a S-demethylation into 7,8-dihydro-8-thio-9-methylpurine (Table II). This reaction proceeded quickly and quantitatively. Also 6-methylthiopurine and 8-methylthiopurine could successfully be demethylated. These reactions are of preparative interest since a similar conversion has only been effected in a few cases, using hydrogen sulphide or phosphorus pentasulphide.[3]

Experimental Section

^1H NMR spectra were obtained with a Varian EM 390 or an Hitachi Perkin-Elmer R-24B (60 MHz) using Me_4Si as internal standard. When measuring in liquid ammonia the sample temperature was ca. -50°C and NH_3 was used as standard. The spectra were converted to the Me_4Si scale by adding 0.95 ppm. Mass spectra and ^{15}N contents were determined on an AEI MS-902 mass spectrometer. UV spectra were obtained with a Beckman Acta C III and a Perkin Elmer 550. Melting points are uncorrected.

Preparation of starting materials

6-Methylthiopurine and 8-methylthiopurine are commercially available. 6-Amino-9-methylpurine[17], 6-methylamino-9-methylpurine[18], 6-dimethylamino-9-methylpurine[18], 6-amino-9-[2'-tetrahydropyranyl] purine[19], 2',3'-O-isopropylidene adenosine[20], 6-amino-2-chloro-9-methylpurine[21], 6-methylthio-9-methylpurine[22], 6-methoxy-9-methylpurine[18] and 8-methylthio-9-methylpurine[23] were prepared according to procedures as described in the literature.

6-Amino-8-deutero-9-methylpurine was obtained by refluxing 6-amino-9-methylpurine in an excess of deuterium oxide.[24] 95% of deuterium was incorporated at position 8, as established by ^1H NMR spectroscopy.

9-Methylpurine (3). Methylation of purine with tetramethylammoniumhydroxide gave

a mixture of 7- and 9-methylpurine.[17] Separation by column chromatography on silica gel using 10% methanol in chloroform as eluent gave 7-methylpurine (yield 24%) and 9-methylpurine (12%).

2,6-Diamino-9-methylpurine (5, X=NH_2)[25]. Method I. 2,6-Dichloro-9-methylpurine was heated with ethanolic ammonia in a sealed tube for 24 h at 160°C. The residue obtained after evaporation of the reaction mixture *in vacuo*, was washed with water and purified by column chromatography on silica gel, using 15% of methanol in chloroform as eluent, yield 50% (recrystallized from water).
Anal.Calcd. for $C_6H_8N_6$: C, 43.89; H, 4.91. Found: C, 44.12; H, 4.97.
Method II. Methylation of 2,6-diaminopurine with tetramethylammoniumhydroxide, sublimation at 260°C and 0.06 mm[17] gave, after purification by column chromatography, 2,6-diamino-9-methylpurine, yield 30%.

6-Amino-2-methoxy-9-methylpurine (5, X=OCH_3). Sublimation of 6-amino-2-methoxypurine with tetramethylammoniumhydroxide (260°C and 0.06 mm)[17] gave a reaction mixture that after purification by column chromatography (silica gel, 10% methanol in chloroform) gave in a yield of 20% 5 (X=OCH_3), being identical with the product prepared according to the procedure described in the literature.[26]

General reduction procedure

15 mL of dry liquid ammonia (destilled from potassium) was condensed and, if necessary, 10 mL of dry diethylether was added. 50 mg of the starting material was introduced while stirring. Small pieces of sodium were added to maintain a blue color during the reaction. The reaction mixture was quenched with ammonium sulphate, the ammonia was evaporated and the residue was extracted with a mixture of chloroform and methanol. Separation of the products could be achieved by column chromatography or preparative T.L.C. with mixtures of chloroform and methanol as eluent. All reaction products being formed and isolated were known compounds except 2-methoxy-9-methylpurine (structure proven by ^{1}H NMR, (Me_2SO-d_6) δ 3.72 (s, NCH_3), 3.93 (s, OCH_3), 8.26 (s) and 8.79 (s), mass spectroscopy and comparison of the UV spectrum with that of 2-ethoxy-9-methylpurine[27](pH 1, λ_{max}= 282 nm; pH 7, λ_{max} = 280 nm), m.p. 140.5-142.5°C, exact mass, calcd. for $C_7H_8N_4O$ 164.0698; found 164.0703).

Acknowledgement

We are indebted to Drs.C.A.Landheer and Mr.W.P.Combé for the mass spectroscopic data, to Mr.A.van Veldhuizen for measuring some NMR spectra and to Mr.H.Jongejan for carrying out the microanalyses.

References

1. Part 28 on σ-adducts. For part 27 see: A.Counotte-Potman, H.C.van der Plas and A.van Veldhuizen, J.Org.Chem. submitted.
2. Part 84 on pyrimidines. For part 83 see: C.Gönczi,Z.Swistun and H.C.van der Plas, J.Org.Chem. in the press.
3. For a review: J.H.Lister in "The Chemistry of Heterocyclic Compounds, Fused Pyrimidines, Part II", D.J.Brown, Ed. Wiley, New York, 1971.
4. S.M.Hecht, B.L.Adams and J.W.Kozarich, J.Org.Chem.41, 2303 (1976).
5. B.L.Adams in "Nucl.Acid.Chem." 2, 615 (1978); L.B.Townsend and R.S.Tipson, Ed. Wiley, New York.
6. Y.Maki, M.Suzuki and K.Ozeki, Tetrahedron Lett. 1199 (1976).
7. Z.Neiman, Experientia 35, 1418 (1979).
8. K.S.V. Santhanam and P.Elving, J.Am.Chem.Soc. 96, 1653 (1974).
9. M.M.Wegner and H.Rapoport, J.Org.Chem. 42, 3065 (1977).
10. V.Nair and S.G.Richardson, J.Org.Chem. 45, 3969 (1980).
11. This behaviour is comparable to the loss of HBr from 1,6-dihydro-6-bromo- or H_2O from 1,6-dihydro-6-hydroxypurine[3] and the loss of ammonia from 1,2,3,6-tetrahydroadenine.[8]
12. N.J.Kos, H.C.van der Plas and A.van Veldhuizen, J.Org.Chem. 44, 3140 (1979).
13. This result is in agreement with literature data describing the debenzylation of some 6-aminopurines without loss of the amino group.[3]
14. E.Y.Sutcliffe and R.K.Robins, J.Org.Chem. 28, 1662 (1963); F.Skoog et al., Phytochemistry 6, 1169 (1967).
15. N.J.Kos and H.C.van der Plas, J.Org.Chem. 45, 2942 (1980).
16. Sodium in liquid ammonia is used to debenzylate benzylthiopurines[3] but it is known that in other compounds methylthio groups can be demethylated in this way. (H.O.House in "Modern Synthetic Reactions", W.A.Benjamin, Inc. Ed. 1972).
17. T.C.Myers and L.Zeleznick, J.Org.Chem. 28, 2087 (1963).
18. R.K.Robins and H.H.Lin, J.Am.Chem.Soc. 79, 490 (1957).
19. R.K.Robins, E.F.Godefroi, E.C.Taylor, L.R.Lewis and A.Jackson, J.Am.Chem.Soc. 83, 2574 (1961).
20. A.Hampton and D.I.Margrath, J.Am.Chem.Soc. 79, 3250 (1957).J.Baddiley, J.Chem.Soc., 1348 (1951).
21. R.Falconer, J.M.Gulland and L.F.Story, J.Chem.Soc., 1784 (1939).
22. D.J.Brown and P.W.Ford, J.Chem.Soc. C., 2620 (1969).
23. R.J.Badger and G.B.Barlin, J.C.S.Perkin Trans II, 1854 (1974).D.J.Brown and S.F.Mason, J.Chem.Soc., 682 (1957).

24. M.Maeda and Y.Kawazoe, Tetrahedron Lett. 1643 (1957).
25. E.C.Taylor, G.P.Beardsley and Y.Maki, J.Org.Chem. 36, 3211 (1971).
26. J.Sepiol, Z.Kazimierczuk and D.Shugar, Z.Naturforsch.C.Biosci 31, 361 (1976).
27. G.B.Barlin, J.Chem.Soc.B, 954 (1967).

6 AN NMR INVESTIGATION OF THE GEOMETRICAL ISOMERISM IN THE ANIONS OF AROMATIC AMINO COMPOUNDS

Nico J.Kos, Koos Breuker, Henk C.van der Plas and Beb van Veldhuizen

The NMR spectra of the anions of 2-, 6- and 8-aminopurines, 2- and 4-aminopyrimidines, 3- and 4-aminopyridazines, aminopyrazine, 2-aminopyridine, aniline and its p-methyl derivative in liquid ammonia containing potassium amide at low temperature show the presence of two geometrical isomers, due to restricted rotation of the deprotonated amino group. The occurrence of coalescence has been observed with aminopyrazine and p-methylaniline.

Introduction

The phenomenon of hindered rotation in N-substituted imines is known for many years; it even occurs in diphenyl ketimine, although only at very low temperature[1]. Recently, geometrical isomers of aromatic amines were found to exist at low temperatures[2-7]. This observation evidences the contribution of mesomeric structures in which the carbon-nitrogen bond has double bond character. Based on these results one has to expect the occurrence of geometrical isomerism in the *anions* of aromatic amines, since delocalization of the negative charge in the aromatic ring will enhance the double bond character of the carbon-nitrogen bond considerably. This has indeed been observed for the anion of N-methyl-2,4,6-trinitroaniline in dimethylsulfoxide (as indicated by the non-equivalence of H-3 and H-5)[8], the anion of 2-(methylamino) pyrimidine (non-equivalence of H-4 and H-6)[9] and in the dianion of adenine (in which two different NH protons are present)[10].
However, ^{1}H NMR spectra of aminopyrazine[11], 2-aminopyridine[11] and several anilines[12] in liquid ammonia containing potassium amide do not indicate the existence of separate isomers. It is possible that in the strong basic medium a fast isomerization takes place, preventing the detection of the separate geometrical isomers. The non-equivalence of hydrogen atoms in a substituent has been found for the $CH_2^{\ominus}$ group in the dianion of 6-methylpurine[13] and in the anions of 4-methylpyrimidine[14], 4-methyl-5-bromopyrimidine[14], 2-methylpyridine[11] and methylpyrazine[11], generated in liquid ammonia containing potassium amide.

Results and Discussion

A. Aminopurines

The ^{1}H NMR spectrum of a solution of adenine in liquid ammonia containing potassium amide shows three sets of two signals (Table I) of which two sets are assigned to aromatic hydrogens and the set of two broad signals to the NH^- group[10].

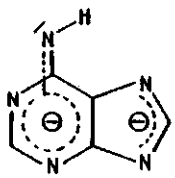
The assignments of two sets of aromatic hydrogens are made by comparison of the ^{1}H NMR spectrum with that of 8-deuterioadenine under identical conditions.

Table I. *^{1}H NMR data of the dianions of some aminopurines in liquid ammonia containing potassium amide at -50°C* [a]

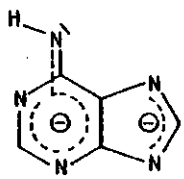
Dianion of	H-2	H-6	H-8	N-H	isomer distribution
adenine (1)	7.53	-	7.37	4.59	65%
	7.64	-	7.34	4.93	35%
8-chloroadenine (3)	7.42	-	-	4.49	65%
	7.53	-	-	4.75	35%
2-aminopurine (4)	-	7.94	7.38	3.83	75%
	-	8.00	7.38	3.69	25%
8-aminopurine (5)	7.18[b]	7.63[b]	-	3.90	-

[a] Chemical shifts in ppm relative to Me_4Si (δ = 0 ppm)

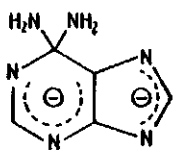
[b] These assignments can also be interchanged



1a



1b



2

The spectra can only be explained if one assumes the presence of dianion 1 (obtained by deprotonation of both the N^9 - H and the NH_2 group) in two distinct geometrical isomers (structures 1a and 1b). Due to delocalization of the negative charge on the amino nitrogen atom over the purine ring the double bond character of the C_6 - N bond is enhanced, resulting in restricted rotation and the formation of 1a and 1b. The presence of both isomers shows that in this basic medium the isomerization and proton exchange are slow (on the NMR time scale). From the ^1H NMR data it could be established that the two geometrical isomers 1a and 1b are present in the ratio 65:35. Which isomer is the more abundant one has not been determined. This ratio is found to be independent of the potassium amide concentration, ranging from 1.5 to 4 equivalents.
The existence of the two geometrical isomers 1a and 1b is confirmed by ^{13}C NMR spectroscopy (Table II). In the decoupled spectrum of a solution of adenine in KNH_2/NH_3 all signals except the ones for C-2 and C-8 appear double. All signals can be assigned unambiguously by comparison of the spectrum with that of deuterioadenine and with literature data.

Table II. ^{13}C NMR data of the dianion of adenine (1) in liquid ammonia containing potassium amide at -50°C[a]

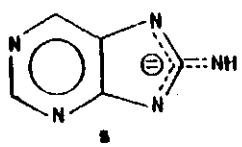
Dianion of	C-2	C-4	C-5	C-6	C-8
adenine (1)	151.6	157.6[b]	123.4[b]	165.1[b]	146.4
	151.6	156.2	124.3	167.7	146.4

[a] Chemical shifts in ppm relative to Me_4Si (δ = 0 ppm)
[b] More abundant isomer

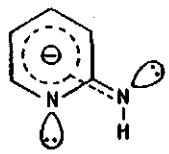
That the double signals for C-2 as well as for C-8 coincide is proven by selective decoupling. Since the ^1H NMR spectral data had shown the isomer ratio 65:35, the set of ^{13}C signals with the greater intensities is assigned to the more abundant isomer.
An attempt to confirm the geometrical isomerism by measuring the coalescence of the ^1H NMR signals with increasing temperature, failed. On allowing the temperature of the solution of the dianion to rise from -50°C up to 15°C in a sealed tube the signals are broadened and the isomer ratio (calculated from the ^1H NMR spectrum) changes from 65:35 to 50:50. We could not measure an average spectrum.

We have ascertained that the signals observed in the NMR spectra are not originated from the σ-adduct 2, possibly formed by attack of the amide ion on C-6 in the monoanion of adenine. Adduct formation in purines is known to occur at position 6[10,13] and adduct formation at a position already occupied by an amino group cannot be excluded either (for example the ring transformation of 4-amino-2-bromoquinoline into 4-amino-2-methylquinazoline[15]). Therefore we reacted adenine with ^{15}N labelled potassium amide. If adduct 2 should be formed, it would lead to incorporation of ^{15}N in recovered adenine. Since in our experiment no ^{15}N label could be found in the recovered adenine (mass spectrometry), we exclude the intermediacy of adduct 2.

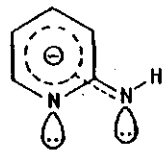
The dianion of 8-chloroadenine (3) and that of 2-aminopurine (4) also show the presence of two isomers in the ^1H NMR spectrum (Table I). For 3 the isomer ratio (65:35) is the same as for adenine ; for 4 a ratio of 75:25 was found. The signal assignment of 4 was based on comparison of the signals with those of 2-amino-8-deuteriopurine.
No double signals are detected in the ^1H NMR spectrum of the dianion of 8-aminopurine (5). That we are dealing with a dianion and not with a monoanion was shown by integration, indicating the presence of only *one* aminohydrogen. This result does not justify the conclusion that only one geometrical isomer is present, since the symmetry in the imidazole part of this dianion may lead to spectral coincidence of both isomers.



5



7a



7b

B. Aminopyrazine, 2-aminopyridine, aminopyridazines and aminopyrimidines
The ^1H NMR spectra of the anion of aminopyrazine (6) as well as of 2-aminopyridine (7) in liquid ammonia containing potassium amide were previously reported without specifying the temperature at which the spectra were measured[11]. The occurrence of geometrical isomers was not mentioned.
We observed, however, that when the ^1H NMR spectra of the anions of these amino compounds are measured at -50°C, the anion 6 exists as a mixture of two geometrical isomers in a ratio of 65:35.

Table III. *^{1}H NMR data of the anions of some monocyclic aromatic amines in liquid ammonia containing potassium amide at -50°C*[a]

Anion of	H-2	H-3	H-4	H-5	H-6	N-H	isomer distribution
aminopyrazine (6)	-	7.25	-	6.57	7.17	4.47	65%
	-	7.14	-	6.63	7.31	4.25	35%
2-aminopyridine (7)	-	5.73	6.70	5.49	7.45	3.90	55%
	-	5.82	6.62	5.42	7.32	4.22	45%
3-amino-6-methyl-pyridazine (8)	-	-	6.12	6.40	-	4.30	70%
	-	-	6.09	6.42	-	3.72	30%
4-aminopyridazine (9)	-	7.76[b]	-	5.88[b]	7.62[b]	4.45	50%
	-	7.90[b]	-	5.72[b]	7.54[b]	4.45	50%
2-amino-4-phenyl-pyrimidine (10)	-	-	-	6.10	7.75	4.70	50%
	-	-	-	6.10	7.75	4.76	50%
4-aminopyrimidine (11)	7.67	-	-	5.81	7.24	4.80	70%
	7.82	-	-	5.78	7.24	4.74	30%
4-methylaniline (12)	5.80	6.40	-	6.32	5.94	2.92	c
2,4-dimethylaniline (13)	-	6.46	-	6.43	6.05	2.70	85%
	-	d	-	6.28	5.84	3.09	15%

[a] Chemical shifts in ppm relative to Me_4Si (δ = 0 ppm)

[b] In these cases it cannot be decided which signals belong to one isomer

[c] Symmetric molecule

[d] Not observable

Raising the temperature gradually changes this ratio to 50:50 at 0°C and finally results in incomplete coalescence at +20°C. Cooling to -50°C restores the 65:35 ratio, proving that the isomers are in thermodynamic equilibrium. Comparison of these results with those of adenine, where at room temperature no coalescence is observed for the dianion of adenine (1) (see section A), indicates that the stabilization of the negative charge in 6 is less than in 1.

The anion of 2-aminopyridine (7) is also present in two geometrical isomers at -50°C (ratio 55:45). The ratio is independent of the concentration of 7 (0.2 - 2 mmol/ml) and of potassium amide (1.5 - 10 equivalents). As no isomerism was reported in the literature[11], it is clear that the ^{1}H NMR spectra must have been measured above the coalescence temperature. The almost equal

concentration of both isomers shows that stabilization of the *syn*-isomer 7a *via* intramolecular hydrogen bonding[5] and destabilization of the *anti*-isomer 7b by repulsion between the two electron pairs[16] is unimportant. The ^{1}H NMR spectrum of the anion of 3-amino-6-methylpyridazine (8) also shows the presence of two geometrical isomers in a ratio of 70:30; thus the preference for one isomer is slightly greater (Table III)than in 7. The methyl group appears as a singlet in each isomer and is therefore probably not deprotonated[11,13,14]. The anion of 4-aminopyridazine (9) is also present in two geometrical forms (Table III). The isomer distribution is 50:50, being the same as for the symmetrical anion of 4-aminopyridine. Thus, the ratio does not change, when a nitrogen atom is introduced in the *meta* position of the anion of 4-aminopyridine.

As already indicated in the introduction, geometrical isomerism has been observed for the anion of 2-(methylamino) pyrimidine[1]. In the present study we find indications for the occurrence of two isomers for the anion of 2-amino-4-phenylpyrimidine (10)(Table III). The spectrum shows two separate NH signals, and two different signals for H-5 (not for H-6). The isomer distribution is 50:50. The results in Table III show further that also for the anion of 4-aminopyrimidine (11) geometrical isomerism exists; the ratio is 70:30.

C. Anilines

The anions of aniline and methylanilines lack the stabilizing ring nitrogens, and therefore may be expected to have a lower rotation barrier, due to a decreased double bond character of the carbon-nitrogen bond. Hence, the coalescence temperature should be lower than in the case of aminopyrazine. In the literature no indication is available for the occurrence of geometrical isomers in the spectra of aniline and methylanilines in liquid ammonia containing potassium amide at +31^{o}C[12]. However, in our study we find that at -50^{o}C the anions of aniline and 4-methylaniline (12) show the presence of geometrical isomers (Table III). As both compounds are symmetric, restricted rotation will be reflected in the non-equivalence of H-2 and H-6 and of H-3 and H-5, but not in the N-H signal. For 12 these different signals for all four ring hydrogen atoms are clearly observed. The simplicity of the spectrum makes this compound suitable for an attempt to measure coalescence.

Figure. Part of the ^1H NMR spectrum of 12 at different temperatures

Indeed, on raising the temperature from -50°C to -27°C coalescence takes place, and at -15°C the spectrum consists of two doublets, representing both equivalent *ortho* and *meta* hydrogens (Figure). The aromatic signals in the spectrum of the anion of aniline at -50°C cannot be simply interpreted, but their complexity suggests non-equivalence of both the *ortho* and the *meta*-hydrogen atoms, and hence restricted rotation of the carbon-nitrogen bond.

The anion of 2,4-dimethylaniline (13) too is found to exist in two isomers, in a ratio of 85:15. This indicates that introduction of a methyl group *ortho* to the amino function has a strong influence on the isomer ratio.

These data show that even in aniline anions, in which no stabilizing substituents or ring nitrogen atoms are present, geometrical isomerism can be observed at -50°C.

Experimental Section

^{13}C and ^1H NMR spectra were obtained with a Varian XL-100-15 spectrometer, equipped with a Varian 620/L16K computer. When measuring in $CDCl_3$, internal Me_4Si was used as standard. When measuring in liquid ammonia the sample temperature was ca. -50°C. Some samples were also measured at higher temperatures in sealed tubes. For ^1H NMR spectra NH_3 was used as standard. The spectra were converted to the Me_4Si scale by adding 0.95 ppm. For ^{13}C NMR spectra Me_3N was used as internal standard. Adding 47.5 ppm converts these spectra to the Me_4Si scale.

Typical ^{13}C NMR spectral parameters were as follows: spectral width 5120 Hz, acquisition time 0.8 s, pulse delay 0-1.2 s, pulse width 10 μs.

Both ^1H and ^{13}C NMR spectra were usually measured on solutions of 0.4-0.6 mmol/ml with 4 equivalents of potassium amide. Isomer ratios were determined by integration of appropriate signals. Mass spectra and ^{15}N contents were determined on an AEI MS-902 mass spectrometer.

6-Amino-8-chloropurine[17], 8-aminopurine[18], 3-amino-6-methylpyridazine[19], 4-aminopyridazine[20], 2-amino-4-phenylpyrimidine[21] and 4-aminopyrimidine[22] were prepared according to the literature. All other compounds were purchased.

Acknowledgement

We are indebted to drs.C.A.Landheer and mr.W.P.Combé for mass spectrometric data.

References

†Dedicated to Professor Tetsuji Kametani for his pioneering work in natural product chemistry and his contribution to the development of heterocyclic chemistry on the occasion of his retirement from the Chair of Organic Chemistry at the Pharmaceutical Institute of Tohoku University at Sendai.

1. J.B.Lambert, W.L.Oliver and J.D.Roberts,J.Am.Chem.Soc. 87, 5085 (1965).
2. J.Almog, A.Y.Meyer and H.Shanan-Atidi, J.Chem.Soc.Perkin II, 451 (1972).
3. B.McConnel and P.C.Seawell, Biochemistry 12, 4426 (1973).
4. M.Raszka, Biochemistry 13, 4616 (1974).
5. G.Dodin, M.Dreyfus and J.E.Dubois, J.Chem.Soc.Perkin II, 438 (1979).
6. G.Barbieri et al., J.Chem.Soc.Perkin II, 330 (1979).
7. J.Riand, M.T.Chenon and N.Lumbroso-Bader, J.Chem.Soc.Perkin II, 1248 (1979).
8. K.L.Servis, J.Am.Chem.Soc. 87, 5495 (1965); ibid. 89, 1508 (1967).
9. J.P.Geerts, H.C.van der Plas and A.van Veldhuizen. Org.Magn.Reson. 7, 86 (1975).
10. N.J.Kos, H.C.van der Plas and A.van Veldhuizen, Recl.Trav.Chim.Pays-Bas 99, 267 (1980).
11. J.A.Zoltewicz and L.S.Helmick, J.Org.Chem. 38, 658 (1973).
12. T.Birchall and W.L.Jolly, J.Am.Chem.Soc. 88, 5439 (1966).
13. N.J.Kos, H.C.van der Plas and A.van Veldhuizen. J.Org.Chem. 44, 3140 (1979).
14. J.P.Geerts, C.A.H.Rasmussen, H.C.van der Plas and A.van Veldhuizen. Recl.Trav.Chim.Pays-Bas 93, 231 (1974).
15. H.J.den Hertog and D.J.Buurman, Recl.Trav.Chim.Pays-Bas 91, 841 (1972).
16. J.A.Zoltewicz and A.A.Sale, J.Am.Chem.Soc. 95, 3928 (1973).
17. R.K.Robins, J.Am.Chem.Soc. 80, 6671 (1958).
18. A.Albert and D.J.Brown, J.Chem.Soc., 2060 (1954).
19. G.B.Barlin and C.Yoot Yap, Austr.J.Chem. 30, 2319 (1977).
20. T.Itai and S.Natsume, Chem.Pharm.Bull. 11, 342 (1963).
21. H.Bredereck et al. Chem.Ber. 98, 1081 (1965).
22. A.Albert, R.Goldacre and J.Phillips, J.Chem.Soc., 2240 (1948).

7 AN NMR INVESTIGATION OF GEOMETRICAL ISORISM IN THE ANIONS OF METHYLAMINOPYRIDINES. ASSIGNMENT OF THE SYN- AND ANTI-ISOMERS

N.J.Kos, J.Breuker, H.C.van der Plas and A.van Veldhuizen

The ^{1}H and ^{13}C NMR spectra of the anions of 4-, 3- and 2-(methylamino) pyridine and some of their *ortho*-methylsubstituted derivatives in liquid ammonia containing potassium amide at -50°C are measured and assigned to the *syn*- and *anti*-isomers. The position of the signals of the *ortho* hydrogen and carbon atoms is discussed.

Introduction

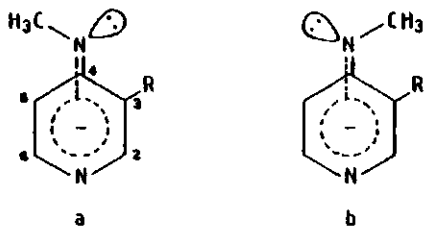
In the literature the ^{1}H NMR spectra of the anions of aminopyrazine[1], 2-aminopyridine[1] and several anilines[2] at 25-31°C are reported. In these studies the existence of geometrical isomers was not mentioned. However, geometrical isomerism has been noticed in the anion of 2,4,6-trinitroaniline[3], and also in our own studies evidence for the occurrence of *syn*- and *anti*-isomers of the anions of amino azaaromatics (2-aminopyridine, 2- and 4-aminopyrimidines, aminopyrazine, 3- and 4-aminopyridazine and 2-, 6- and 8-aminopurines) has been obtained[4,5,6].

This phenomenon has been ascribed to an enhanced double bond character of the exocyclic carbon-nitrogen bond, leading to restricted rotation[4,5]. In aniline and methylanilines restricted rotation has been found at -50°C[4]. It has been reported that the anion of 2-(methylamino)pyrimidine also shows geometrical isomerism, as appears by the non-equivalency of H-4 and H-6[5]. In order to investigate the generality of this phenomenon in (methylamino)aza heteroarenes we measured ^{1}H and ^{13}C NMR spectra of the anions of 4-, 3- and 2-(methylamino)pyridine. Since the presence of a methyl substituent *ortho* to the methylamino group has an important influence upon the *syn-anti* ratio, which can provide us with an important clue to *syn-anti* assignment, we included in our study the NMR spectroscopy of the anions of 3-methyl-4-(methylamino)-, 4-methyl-3-(methylamino)- and 3-methyl-2-(methylamino) pyridine.

Results and Discussion

A. 4-(methylamino)pyridines

The ^{1}H NMR spectrum of the anion of 4-(methylamino)pyridine (1) measured in liquid ammonia containing potassium amide at -50°C shows just as the neutral compound[7] geometrical isomerism. This phenomenon is revealed by the appearance of separate signals for H-3 and H-5 and for H-2 and H-6, showing that these hydrogens are not identical.



a b

1: R = H
2: R = CH_3

Also the ^{13}C NMR spectrum shows separate signals for both C-3 and C-5 and for C-2 and C-6 carbon atoms. In order to be able to assign the ^{1}H NMR signals to the respective atoms in each of the isomers a and b we prepared the anion of 3-methyl-4-(methylamino)pyridine (2) and compared the spectra of 1 and 2. We observed that the ^{1}H NMR spectrum of 2 only consists of a singlet (H-2) and two doublets (H-5 and H-6, Table I); there is no indication for the existence of two isomeric forms. It can be questioned whether this is due to the fact that this spectrum is an average of the two structures, isomerizing fast on the NMR time scale. However, we feel that this is not probable on the following grounds: i. in 3-methyl-4-(dimethylamino)pyridine the mesomeric interaction between the pyridine ring and the dimethylamino group is not seriously hindered by an *ortho* methyl substituent[8]; i.i. the related compound 2-methyl-4-nitro-N-methylaniline is present in only *one* form at temperatures between -150°C and -50°C[7]. i.i.i. 4-(methylamino)pyridine (thus not the anion) undergoes coalescence at about -60°C.

Since in our study we are dealing with anions, we have to expect higher coalescence temperatures, therefore the two isomeric forms 2a and 2b should be observable at the temperature we used (-50°C).

Table I. ^{1}H *NMR data of the anions of N-methylaminopyridines in liquid ammonia containing potassium amide at -50°C*[a,b]

Anion of		H-2	H-3	H-4	H-5	H-6	NCH_3	isomer distr. %
4-(methylamino)-pyridine	(1a)	7.22	5.89	-	5.60	7.43	2.54	50:50
3-methyl-4-(methyl-amino)pyridine[c]	(2a)	7.25	-	-	5.62	7.47	2.66	100
3-(methylamino)-pyridine	(4a)	7.50	-	5.76	[d]	[d]	2.50	80
	(4b)	7.03	-	[d]	[d]	6.84	2.60	20
4-methyl-3-(methyl-amino)pyridine[e]	(5b)	6.95	-	-	6.47	6.84	2.70	100
2-(methylamino)-pyridine	(6a)	-	5.81	6.57	5.35	7.50	2.62	40[g]
	(6b)	-	5.58	6.96	5.57	7.57	2.54	60[g]
3-methyl-2-(methyl-amino)pyridine[f]	(7a)	-	-	6.50	5.35	7.43	2.71	100

[a] Chemical shifts in ppm relative to Me_4Si (δ = 0 ppm)

[b] Coupling constants: $J_{2,3}$= 6 Hz, $J_{2,4}$= 3 Hz, $J_{3,4}$= 8-8.5 Hz, $J_{3,5}$= 1.5-2.5 Hz, $J_{4,5}$= 6-8 Hz, $J_{4,6}$= 1.5-2.5 Hz, $J_{5,6}$= 4-6 Hz.

[c] The methyl group at C-3 is found at 1.81 ppm

[d] These signals are present in a complex region between 6.4 and 6.7 ppm

[e] The methyl group at C-4 is found at 1.87 ppm

[f] The methyl group at C-3 is found at 1.76 ppm

[g] Ratio after equilibration at +25°C

In fact the temperature range between -80 and +10°C showed only one set of NMR signals. This leads to the conclusion that the spectrum of anion 2 measured under these conditions can only be explained by the presence of *one* isomer, *i.e.* the one in which the methyl of the methylamino group and the *ortho* methyl are directed away from each other, due to repulsion (structure 2a).

Table II. ^{13}C *NMR data of N-methylaminopyridines in* $CDCl_3$ *at 35°C and of their anions in liquid ammonia containing potassium amide at -50°C*[a]

Compound	C-2	C-3	C-4	C-5	C-6	NCH_3
4-(methylamino)-pyridine neutral	149.8	107.3	154.9	107.3	149.8	29.2
anion(1a)	146.1	115.0	163.0	102.6	149.5	36.5
1,2,3,4-tetrahydro-1,6-naphthyridine[9]						
neutral	137.7[b]	117.7	156.8	108.6	137.3[b]	
anion (3)	146.0	c	c	110.6	146.0	
3-(methylamino)-pyridine neutral	135.5	146.0	118.1	124.0	137.7	30.0
anion(4a)	143.6	159.2	107.4	124.8	122.6	37.3
anion(4b)	129.5	c	123.0[b]	122.3[b]	125.1[b]	37.3
4-methyl-3-(methyl-amino)pyridine						
neutral[d]	130.0	144.7	132.2	125.1	137.3	30.4
anion(5b)[e]	127.0	156.3	130.2	122.7	124.5	37.1
2-(methylamino)-pyridine neutral	160.0	106.3	137.6	112.7	148.2	29.0
anion(6a)	166.7	114.5	132.2	99.1	149.1	35.5
anion(6b)	168.6	102.0	136.2	100.1	149.6	36.6

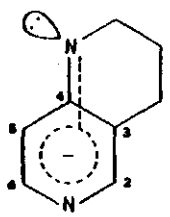
[a] Chemical shifts in ppm relative to Me_4Si (δ = 0 ppm)
[b] These signals may also be interchanged
[c] These signals could not be observed
[d] The methyl group at C-4 is found at 17.2 ppm
[e] The methyl group at C-4 is found at 19.2 ppm

In the ^1H NMR spectrum of anion 1 the signals of H-3 and H-5 are well separated (δ = 5.89 and 5.60 ppm relative to Me_4Si, Table I), and the chemical shift of one of these is very close to that of H-5 in anion 2a (5.62 ppm). Also the chemical shifts of the two signals of H-2 and H-6 of 1 (7.22 and 7.43 ppm) are very similar to those of H-2 and H-6 of 2a (7.25 and 7.47 ppm). These close

resemblances make it evident to assign the signals in the spectrum of 1 as indicated in Table I. From this result it appears that the *ortho* hydrogen atom *syn* oriented to the nitrogen lone pair (H-3) resonates more downfield than the *anti* hydrogen (H-5). It should be noted that we have represented the formulas throughout this paper with an sp^2 lone pair, with a *p*-orbital in conjugation with the pyridine ring. We realize that this is only an approximation of the two electron pairs on the nitrogen, since the negative charge is not fully delocalized in the aromatic ring.

With the assignments of the ^1H NMR spectra of 1 and 2a we are able to interpret the ^{13}C NMR spectra of anion 1 by selective decoupling experiments. The results are that the *ortho* hydrogen resonating at lower field (H-3) is bound to the *ortho* carbon atom at lower field (C-3), and that the hydrogen at higher field (H-5) is bound to carbon at higher field (C-5). The same relationship is found for the H-2, H-6 hydrogen and C-2 and C-6 carbon atoms. These results lead to the assignment as given in Table II.

This ^{13}C assignment of anion 1 is confirmed by the spectrum of the anion of the tetrahydro-1,6-naphthyridine (3)[9], which may be regarded as a model for anion 2b of 3-methyl-4-(methylamino)pyridine in which the *ortho* hydrogen (H-5) and the lone pair are in the *syn* orientation, and the methyl groups directed to each other. The *ortho* carbon atom C-5 in anion 3 being in *syn* orientation to the lone pair exhibits a downfield shift compared to the neutral compound. A downfield shift is also found for C-3 in anion 1a, being in *syn* position relative to the nitrogen lone pair.



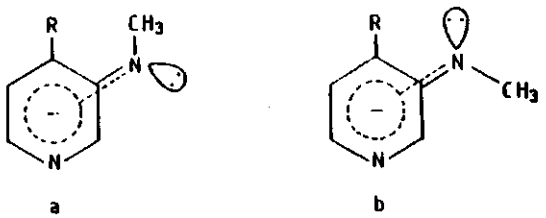
3

All results indicate that in anion 1 the *ortho* carbon atom in the *syn* position relative to the lone pair resonates at a lower field than the *ortho* carbon in the *anti* position. Thus, this is in analogy to what has been found for the *ortho* hydrogen atoms.

B. 3-(methylamino)pyridines

In the ^1H NMR spectrum of the anion of 3-(methylamino)pyridine (4) H-2 appears as two well-separated signals (7.50 amd 7.03 ppm, Table I); only one distinct signal for H-4 is observed at 5.76 ppm. Integration shows that this signal

belongs to the same isomer as the H-2 signal at 7.50 ppm.



4: R = H
5: R = CH_3

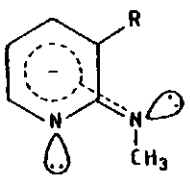
This isomer takes about 80% of the mixture (calculated from the H-2 signals) and the percentage is independent of the temperature (varied from -50 to +20°C). Unfortunately, we could not assign the signals in the spectrum between 6.4 and 6,7 ppm, due to its complexity. Combined with the data from the spectrum of the anion of 4-methyl-3-(methylamino)pyridine (5), being present only in conformation 5b (for the same reasons as mentioned in section A for anion 2) we were able to assign the observed signals of 4. The chemical shifts of the H-2 and H-6 signals of 5b (6.95 and 6.84 ppm respectively) are very close to the corresponding signals of the minor isomer of 4 (7.03 and 6.84 ppm, Table I). Hence the less abundant isomer should have structure 4b. This means that *ortho* hydrogen H-2 in anion 4a, being in a *syn* orientation with respect to the nitrogen lone pair is less shielded than H-2 in 4b (*anti*); also H-4 in *syn* orientation between 6.4 and 6.7 ppm (anion 4b) is found more downfield than H-4 in 4a. This is in accordance with the results in section A for anion 1.
In the ^{13}C NMR spectra the resonance line of C-2 in anion 5b at 127.0 ppm is close to that of C-2 in 4b (129.5 ppm). From selective decoupling experiments with 4 it became clear that the lower field *ortho* hydrogens (H-2 in 4a and H-4 in 4b) are bound to the downfield *ortho* carbons C-2 and C-4 respectively. Thus, the *ortho* carbon atom C-2 which is in the *syn* position to the nitrogen lone pair, resonates at lower field than C-2 in the *anti* position; for C-4 the same relationship is found. These results are in agreement with those found for anion 1 (see section A).

C. 2-(methylamino)pyridines

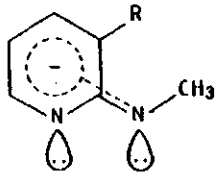
In the NMR spectra of the anion of 2-(methylamino)pyridine (6) again two isomers can be observed. The ratio of these isomers is found to vary between 60:40 and 40:60 at -50°C. When the temperature was allowed to rise from -50°C to 25°C (followed by measurement of the NMR spectrum at -45°C) a reproducible ratio was found (40:60). Apparently we are dealing with a change from a kinetic distribution to the thermodynamic equilibrium.

For the assignment of the spectra of the thermodynamically controlled mixture we applied the same criteria as applied in sections A and B. The ^{1}H NMR spectrum of the minor isomer is similar to the one of the anion of 3-methyl-2-(methylamino)pyridine (7, Table I), being expected to exist in conformation 7a only. Thus, the minor isomer has conformation 6a and the more favoured structure is 6 b. The predominance of 6b is unexpected since repulsion of the electron pairs on the NCH_3^- group and on the ring nitrogen would favour the formation of isomer 6a. The preference for 6 to be present in conformation 6b may be caused by complex formation between the potassium cation and the electron pairs of 6b. In order to establish whether complexation is operative, 18-crown-6-ether, being effective in complexation of potassium cations, was added to the solution. With increasing concentration of crown-ether the signals of 6b decrease, and even disappear in favour of 6a. Furthermore, when instead of potassium amide caesium amide is used as base, a change of the isomeric ratio from 40:60 to 55:45 in favour of conformer 6a has been observed. These results suggest the complexation of 6b with the potassium cation is the dominating factor determining the isomer distribution. The larger size of the caesium ion makes this complexation less efficient. The preferred formation of 6a under kinetic control is not due to a preference of the neutral compound for a conformation like 6a, because a solution of 2-(methylamino)pyridine in methanol does not show isomerism on cooling to -110°C.

In the major isomer 6b,H-3 (*anti* to the lone pair) is more shielded than in 6a. As concluded from selective decoupling experiments in the ^{13}C NMR spectrum the resonance of C-3 *syn* to the lone pair (6a) is at lower field (114.5 ppm) than in the *anti* position (102.0 ppm). This is in agreement with the results obtained with anions 1 and 4 (see sections A and B).



a

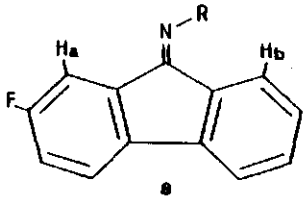


b

α: R = H
γ: R = CH_3

Conclusion

In the foregoing we demonstrated that in all three isomeric N-methylaminopyridine anions the *ortho* hydrogen *syn* oriented to the amino lone pair is less shielded than in *anti* position. The *syn* hydrogen is thus found at a lower field than the *anti* hydrogen. The same effect has been reported for aromatic hydrogens in arylketimines[10,11] and in fluorene derivatives (8)[12]. In the latter compounds assignment was based on the H-F coupling. The difference between the chemical shifts of H_a and H_b in 8 can be as large as 1.3 ppm, with H_a more downfield[12]. Also in all three N-methylaminopyridine anions the *ortho* carbon in *anti* orientation relative to the lone pair resonates at higher field. This upfield shift may be due to steric compression by the N-methyl group[11,13,14]. These experimental results confirm the theoretical calculations which Lunazzi et al. used to assign the *ortho* carbon atoms in N-methylaniline[15].



8
R = alkyl

This neutral compound exists in two isomeric forms at -130°C. For the *meta* carbon atoms was found that the carbon on the *same* side of the molecule as the N-methyl group was associated with the lower field absorption. This was confirmed by our experiments.

Experimental

The procedures followed to obtain the ^{1}H and ^{13}C NMR spectra have been described previously[4]. All compounds were synthesized according to known procedures (4-(methylamino)pyridine[16], 3-methyl-4-(methylamino)pyridine[17], 1,2,3,4-tetrahydro-1,6-naphthyridine[18], 3-(methylamino)pyridine[19], 4-methyl-3-(methylamino)pyridine[20], 2-(methylamino)pyridine[16] and 3-methyl-2-(methylamino)-pyridine[21]).

Acknowledgement

We are indebted to all those, who synthesized the compounds used in this study, to Dr.M.J.A.de Bie (State University Utrecht) and Dr.J.F.J.Engbersen for helpful discussions and to Mr.M.van Dijk for his assistance.

References

1. J.A.Zoltewicz and L.S.Helmick, J.Org.Chem. 38, 658 (1973).
2. T.Birchall and W.L.Jolly, J.Am.Chem.Soc. 88, 5439 (1966).
3. K.L.Servis, J.Am.Chem.Soc. 87, 5495 (1965); ibid. 89, 1508 (1967); M.R. Crampton, B.Gibson and R.S.Matthews, Org.Magn.Reson. 13, 455(1980).
4. N.J.Kos, J.Breuker, H.C.van der Plas and A.van Veldhuizen, Heterocycles, in the press.
5. J.P.Geerts, H.C.van der Plas and A.van Veldhuizen, Org.Magn.Reson.7, 86 (1975).
6. N.J.Kos, H.C.van der Plas and A.van Veldhuizen, Recl.Trav.Chim.Pays-Bas 99, 267 (1980).
7. L.Lunazzi, C.Magagnoli and D.Macciantelli, J.Chem.Soc.Perkin Trans.II, 1704 (1980).
8. Z.Proba and K.L.Wierzchowski, J.Chem.Soc.Perkin Trans II, 1119 (1978).
9. In order to make it easier to compare the data with the other compounds, the numbering of the atoms is chosen as is shown in structure 3.
10. H.Ahlbrecht, Tetrahedron Lett., 4491 (1972).
11. S.Arseniyades, A.Laurent and P.Mison, Bull.Soc.Chim.France II, 233 (1980).
12. R.Knorr, A.Schnegg, E.Lattke and E.Räpple, Chem.Ber. 112, 3490 (1979).
13. G.C.Levy and G.L.Nelson, J.Am.Chem.Soc. 94, 4897 (1972).
14. C.A.Bunnell and P.L.Fuchs, J.Org.Chem. 42, 2614 (1977).
15. L.Lunazzi, C.Magagnoli, M.Guerra and D.Macciantelli, Tetrahedron Lett., 3031

(1979).
16. S.Yamada and Y.Kikugawa, Chem.Ind.(London), 1325 (1967).
17. J.M.Essery and K.Schofield, J.Chem.Soc., 4953 (1960).
18. W.L.F.Armarego, J.Chem.Soc. C. 377 (1967).
19. E.Plazek, A.Marcinikow and Ch.Stammer, Roczniki Chem. 15, 365 (1935); C.A. 30, 1377 (1936).
20. Y.Sawa and R.Maeda, C.A. 62, P.14635 (1965).
21. M.M.Robison and B.L.Robison, J.Am.Chem.Soc. 77, 6554 (1955).

8 AN NMR INVESTIGATION OF GEOMETRICAL ISOMERISM IN THE ANIONS OF AROMATIC AMINO COMPOUNDS. THE "EFFECTIVE SIZE" OF A LONE ELECTRON PAIR

Koos Breuker, Nico J.Kos, Henk C.van der Plas and Beb van Veldhuizen

The ^{1}H and ^{13}C NMR spectra of the anions of 4-, 3- and 2-aminopyridine, 4-aminopyrimidine and some of their methyl derivatives in liquid ammonia containing potassium amide at -50°C are measured and assigned to the *syn* and *anti* isomers. The influence of an *ortho* methyl substituent on the isomer ratio gives an indication for the "effective size" of the nitrogen lone electron pair, which in these anions appears to be larger than the hydrogen in the NH^- group. Comparison of the ^{13}C NMR spectra with those of (methylamino)pyridines reveals a great difference in the effect of the orientation of the NH^- and of the NCH_3^- group on the chemical shifts of the *ortho* carbon atoms. In the aminopyridine anions the carbons *syn* with respect to the nitrogen lone pair resonate at higher field than in the *anti* position, whereas in the (methylamino)pyridine anions a reversed relationship was found.

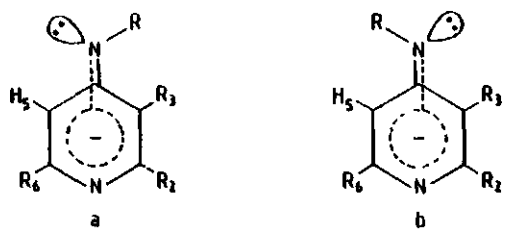
Introduction

A recent NMR spectroscopic study of the anions of some aromatic amino compounds in liquid ammonia containing potassium amide at -50°C has unequivocally proved the occurrence of geometrical isomerism in these systems[1]. This phenomenon has been ascribed to an enhanced double bond character of the exocyclic carbon-nitrogen bond, leading to restricted rotation. From a ^{1}H and ^{13}C NMR study of the anions of 2-, 3- and 4-(methylamino)pyridines, allowing the assignment of the *syn* and *anti* isomers[2], it appeared that the *ortho* hydrogen and carbon atoms being *syn* oriented to the electron pair of the methylamino group all resonate at a lower field than the hydrogen and carbon atoms in the *anti* position. In continuation of this work we studied the ^{1}H and ^{13}C NMR spectra of the anions of 4-, 3- and 2-aminopyridine and some of their C-methyl derivatives, and established the assignment of the signals to either the *syn* or the *anti* isomer.

Results and Discussion

A. 4-Aminopyridines

In the ^1H NMR spectra of the anions of 4-aminopyridine (1) and 4-amino-2,6-dimethylpyridine (2), measured in liquid ammonia containing potassium amide at -50°C, different signals appear for all aromatic hydrogen atoms (Table I).



	R	R_2	R_3	R_6
1:	H	H	H	H
2:	H	CH_3	H	CH_3
3:	H	H	CH_3	H
4:	CH_3	H	H	H

The non-equivalency of the H-3 and H-5 and of the H-2 and H-6 is a result of restricted rotation around the exocyclic carbon-nitrogen bond. Also for the anion of 4-amino-3-methylpyridine (3) geometrical isomers are observed in the ^1H NMR spectra as appears from two signals for each H-2, H-5 and H-6 (Table I). For anions 1 and 2 the isomeric ratio is, of course, 50:50, due to their symmetry; for anion 3 an isomeric ratio of 75:25 is found (determined by integration of appropriate proton signals), showing that a methyl substituent in *ortho* position to the NH^- group has a considerable influence on the isomer distribution[1]. When the solution containing 3 was allowed to stand for one day at room temperature in a sealed tube, the spectrum did not change. This shows that the *syn* and *anti* isomers of 3 are in thermodynamic equilibrium.

To decide which signals belong to either the *syn* or *anti* structure of the anions we used both ^1H and ^{13}C NMR spectroscopic data (Tables I and II) and applied two criteria for discerning these structures. The first one is the well-known dependence of the coupling constant $^3J_{^{13}C-NH}$ on the geometry of the system[3]. When using the $^3J_{^{13}C-NH}$ between the hydrogen of the anionic amino group and the carbon atom in *ortho* position, the coupling constant is larger for the *anti* than for the

syn structure[3]. The second criterion is the chemical shift of the *ortho* hydrogens. In a previous paper it was unequivocally established that an *ortho* hydrogen, being in a position *syn* relative to the lone pair of an anionic methylamino group is more deshielded than in the *anti* position and thus appears at lower field[2]. We can use this result in the aminopyridine anions, since the shielding of the *ortho* hydrogen in these anions will be primarily determined by electric field effects caused by the lone pair orientation and in less extent by the N-H or $N-CH_3$ group. The term "lone pair" is used for the two electron pairs on the amino nitrogen atom. The formulas throughout this paper might suggest an sp^2 lone pair, with a p-orbital in conjugation with the pyridine ring. We realize that this is only an approximation, since the negative charge is not fully delocalized in the aromatic ring. Considering the spectra of anion 3, we observed that in the predominant isomer $^3J_{13_{C-NH}}$ for C-5 is larger (15 Hz compared to 8 Hz, Table II) and that the H-5 signal is at lower field than H-5 in the minor isomer (Table I). Based on these two criteria we reached the conclusion that isomer 3a is the predominant one. This result seems to indicate that the "effective size" of the electron pair on the NH^- group is *larger* than that of the hydrogen atom of NH^-, leading to a preference for 3a in which the proton is near the *ortho* methyl substituent. A somewhat surprising result, since the sp^2 electron pair in pyridine and comparable compounds[4,5,6] as well as the sp^3 electron pair in, for instance, piperidine[7] are found to be "smaller" than a hydrogen atom. However, in the anions, being investigated in this study, two distinct differences have to be taken into consideration in comparison with the systems, mentioned in the literature: i. in liquid ammonia solvation takes place and probably makes the electron pair effectively larger than a hydrogen[8]. i.i. we are dealing with an electron pair in an anionic amino group and theoretical calculations have shown that the size of an electron pair in the series NH_3- NH_2^-- NH^{2-} strongly increases[9].

It cannot be excluded that isomer 3a is better solvated than isomer 3b, since solvation of the electron pair in 3b may be hindered by the *ortho* methyl substituent, leading to destabilization. A complication also arises from the possibility that there is an electronic preference for one of the isomers[10].

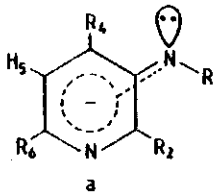
It is interesting to notice that the coupling constants $^3J_{13_{C-NH}}$ for C-5 of 3a (15 Hz) and 3b (8 Hz) are not smaller than for C-3 and C-5 of 1 and 2 (Table II). $^3J_{13_{C-NH}}$ strongly depends on the configuration and will be sensitive to rotation of the amino group[3]. Apparently the *ortho* methyl substituent is not able to push

the amino group out of the plane of the aromatic ring[11]. For the interpretation of the ^{1}H and ^{13}C NMR signals of the anions 1 and 2 the same two criteria are used as mentioned above (Tables I and II).

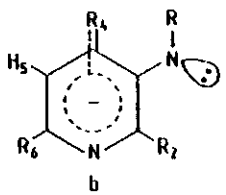
The assignments of the signals for C-2 and C-6 in both anions may be interchanged. Comparison with the spectra of 3 did not make a definitive assignment of C-2 and C-6 possible either. Comparison of the ^{13}C NMR spectra of the now firmly established structures 1a, 2a and 3a with that of the anion of 4-(methylamino)pyridine (4a,Table II) showed the interesting feature that in 4a the signal of the *ortho* carbon atom *anti* relative to the lone pair (C-3) is found at higher field than C-5[2], while in 1a, 2a and 3a the higher field signal has to be ascribed to the carbon atom in the *syn* position (C-5). The anomalous behaviour of C-3 may be due to steric compression in 3a.

B. 3-Aminopyridines

In the ^{1}H NMR spectrum of the anion of 3-aminopyridine (5) two isomers can be discerned in a ratio 60:40. For the signal assignment of the hydrogens in the two isomeric configurations (5a and 5b) we applied the same two criteria as mentioned in section A. Since establishment of the magnitude of the $^{3}J_{^{13}C(4)\text{-}NH}$ is disturbed by coupling of C-4 with H-6, we measured $^{3}J_{^{13}C(4)\text{-}NH}$ in the anion of 3-amino-6-methylpyridine (6)[12]. Anion 6 gives the same isomeric ratio as 5, *i.e.* 60:40. Application of the two criteria gives for the more abundant isomer of 6 the following results (Tables I and II) : i. $^{3}J_{^{13}C\text{-}NH}$ for C-4 is 13 Hz and smaller for C-2; i.i. the *ortho* H-4 is observed at lower field than H-4 in the minor isomer.



a



b

	R	R_2	R_4	R_6
5:	H	H	H	H
6:	H	H	H	CH_3
7:	H	CH_3	H	H
8:	H	H	CH_3	H
9:	CH_3	H	H	H

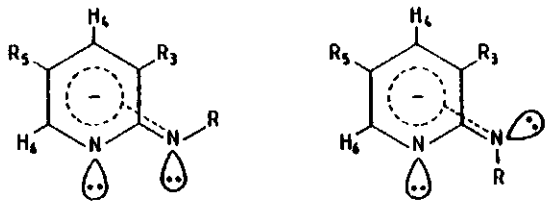
These results lead to the conclusion that isomer 6a is predominant over 6b. This is confirmed by the chemical shift of H-2, being more upfield in 6a than H-2 in 6b, and the magnitude of $^{3}J_{^{13}C\text{-}NH}$ of the less abundant isomer, being 8 Hz for C-4. The same result is obtained for 5.

From the chemical shifts of H-4 in the anion of 3-amino-2-methylpyridine (7) it can be concluded that the preferred isomer has configuration a. The isomer ratio has slightly changed in favour of structure a (70:30). Thus, it is evident that the presence of a methyl group at position 2 promotes the formation of configuration a. Based on the chemical shift of H-2 in the anion of 3-amino-4-methylpyridine (8, Table I), being at lower field in the more abundant isomer, 8b is the favoured isomer. From these results it is evident that a methyl substituent *ortho* to the anionic amino group causes a preference for the isomer in which the amino hydrogen is directed to the methyl group. This indicates, that the steric requirement of the electron cloud on the anionic amino group can be considered as "effectively larger" than the amino hydrogen. This conclusion is in agreement with the one reached in section A.

Considering the ^{13}C NMR spectra of anion 6 it appears that the carbon atom *syn* to the lone pair (C-4, $^{3}J_{^{13}C\text{-}NH}$ = 13 Hz) resonates at higher field than C-4 in the *anti* position ($^{3}J_{^{13}C\text{-}NH}$ = 8 Hz). An analogous difference is observed for C-2. When we compare this result with that of our previous study concerning the anion of 3-(methylamino)pyridine (9) we see that this relationship is reversed: both in 9a and 9b the *ortho* carbon atoms *syn* to the lone pair are found more downfield than in the *anti* position[2]. In section A an analogous difference between 4-aminopyridine and 4-(methylamino)pyridine was found. A second interesting difference between 5 and 9 concerns the isomer ratio. Anion 9 exists mainly (80%) in configuration b, whereas for 5 structure a is predominant.

C. 2-Aminopyridines

We have already reported that the anion of 2-aminopyridine (10) exists in two isomeric forms (ratio 55:45)[1]. This ratio was shown to be independent of the concentration of both 10 and potassium amide[1]. To facilitate the determination of $^{3}J_{^{13}C\text{-}NH}$ we measured the ^{13}C NMR spectrum of the anion of 2-amino-5-methylpyridine (11). The isomeric forms of 11 are present in the same ratio as in 10. For the *syn- anti* assignment we used again the same two criteria as discussed already, *i.e.* $^{3}J_{^{13}C\text{-}NH}$ for C-3 and the chemical shift difference of H-3.



a b

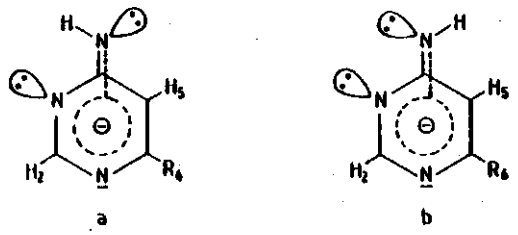
	R	R_3	R_5
10:	H	H	H
11:	H	H	CH_3
12:	H	CH_3	H
13:	CH_3	H	H

From the results shown in Tables I and II we came to the conclusion that conformer 10a, in which the two electron pairs are in the *syn* orientation is slightly preferred, although by repulsion of the electron pairs on the two nitrogen atoms and intramolecular hydrogen bonding[13] 10b would be expected to be favoured. The observed predominancy of 10a may be ascribed to stabilization as a result of complexation of the potassium cations with both lone pairs. Some evidence for this hypothesis was obtained from the observation that the amount of 10a decreases and even disappears in favour of 10b on addition of 18-crown-6-ether, a compound known to complexate with potassium cations. Using cesium amide instead of potassium amide also slightly favours 10b (ratio 35:65).These results are analogous to the behaviour of the anion of 2-(methylamino)pyridine (13). In the anion of 2-amino-3-methylpyridine (12) the *syn-anti* assignment cannot be based on the position of a hydrogen atom *ortho* to the amino group, but comparison of the chemical shifts of H-4, H-5 and H-6 in the anions 10, 11 and 12 indicates that the predominant structure is 12a. This is confirmed by the change of the isomeric ratio in favour of 12b, when using 18-crown-6-ether or adding cesium amide instead of potassium amide. The preference for 12a is stronger (70:30) than for 10a and 11a (55:45), and does not alter when the temperature is allowed to rise to 20°C. This result can again be explained in terms of the electron pair being "larger" than a hydrogen atom. Considering the position of the C-3 signal in the ^{13}C NMR spectrum of anions 10 and 11, it is evident that this *ortho* carbon atom in the *syn* position relative to the lone pair ($^3J_{^{13}C\text{-}NH}$ = 13 Hz) is observed at higher field than in the *anti* position

($^3J_{13C-NH}$ = 6 Hz). In anion 13 this relationship is reversed. This is in agreement with the results in sections A and B.

D. 4-Aminopyrimidines

We have already reported that the anion of 4-aminopyrimidine (14) gives two isomeric conformations in the ratio of 70:30 (Table I)[1]. This ratio does not change on standing at room temperature for an hour, so we are dealing with a thermodynamic equilibrium. For the *syn-anti* assignment we can use the signals H-5, C-5 and $^3J_{13C-NH}$. In the more abundant isomer H-5 appears at lower field and C-5 at higher field, while $^3J_{13C-NH}$ is 12 Hz compared with 7 Hz for the other isomer, showing that 14a is the predominant isomer.



a b

14: $R_6 = H$
15: $R_6 = CH_3$
16: $R_6 = C_6H_5$

This conclusion is further substantiated by the observation that on addition of 18-crown-6-ether the signals of the minor isomer 14b, which is probably stabilized by complexation with the potassium cation, disappear. Also when cesium amide is used instead of potassium amide we find less of 14b.
From the ^1H NMR spectra of the anions of 4-amino-6-methylpyrimidine (15) and 4-amino-6-phenylpyrimidine (16) it is evident that also these anions are preferably present in configuration a (Table I). Apparently a methyl or phenyl group in position 6 does not significantly influence the isomer ratio.
The interesting fact that the anions 14, 15 and 16 prefer the isomeric form in which the two electron pairs are in the *anti* orientation, while the anion of 2-aminopyridine (10) slightly prefers the *syn* structure, is possibly caused by different delocalization pattern of the negative charge. This may be due to the fact that in 4-aminopyrimidine (14b) a part of the negative charge is

Table I. ^{1}H *NMR data of the anions of aminopyridines and aminopyrimidines in liquid ammonia containing potassium amide at -50°C*[a,b]

Anion of		H-2	H-3	H-4	H-5	H-6	CH_3	NH	isomer distribution (%)
4-aminopyridine	1a	7.26	5.66	-	5.82	7.18	-	4.00	50:50
4-amino-2,6-dimethylpyridine	2a	-	5.46	-	5.62	-	1.91 1.95	3.86	50:50
4-amino-3-methylpyridine	3a	7.22	-	-	5.89	7.27	1.74	3.84	75
	3b	7.20	-	-	5.64	7.14	1.79	4.12	25
4-(methylamino)pyridine	4a	7.43	5.60	-	5.89	7.22	2.54	-	
3-aminopyridine	5a	7.21	-	6.20	6.39	6.70	-	3.19[c]	60
	5b	7.39	-	6.03	6.47	6.70	-	3.15[c]	40
3-amino-6-methylpyridine[12]	6a	7.15	-	6.16	6.24[c]	-	2.03	2.92	60
	6b	7.32	-	6.00	6.33[c]	-	2.03	2.84	40
3-amino-2-methylpyridine	7a	-	-	6.23	6.47[c]	6.71	2.02	2.90	70
	7b	-	-	5.96	6.39[c]	6.65	2.02	3.33	30
3-amino-4-methylpyridine	8a	7.15	-	-	6.40	6.69	1.87	3.32	35
	8b	7.41	-	-	6.45	6.74	1.82	2.92	65
3-(methylamino)pyridine	9a	7.03	-	d	d	6.84	2.60	-	20
	9b	7.50	-	5.76	d	d	2.50	-	80
2-aminopyridine	10a	-	5.73	6.70	5.49	7.45	-	3.90	55
	10b	-	5.82	6.62	5.42	7.32	-	4.22	45
2-amino-5-methylpyridine	11a	-	5.70	6.55	-	7.25	1.85	3.7	55
	11b	-	5.82	6.52	-	7.12	1.85	4.0	45
2-amino-3-methylpyridine	12a	-	-	6.68	5.51	7.42	1.79	3.74	70
	12b	-	-	6.55	5.40	7.23	1.79	4.39	30
2-(methylamino)pyridine	13a	-	5.58	6.96	5.57	7.57	2.54	-	60
	13b	-	5.81	6.57	5.35	7.50	2.62	-	40
4-aminopyrimidine	14a	7.67	-	-	5.81	7.24	-	4.80	70
	14b	7.82	-	-	5.78	7.24	-	4.74	30

4-amino-6-methylpyrimidine	15a	7.54	-	-	5.58	-	1.83	4.73	70
	15b	7.70	-	-	5.52	-	1.83	4.53	30
4-amino-6-phenylpyrimidine	16a	7.76	-	-	6.34	-	-	5.05	75
	16b	e	-	-	6.26	-	-	4.95	25

[a] chemical shifts relative to Me_4Si (δ = 0 ppm)

[b] coupling constants: $J_{2,3}$= 6 Hz, $J_{2,4}$= 3 Hz, $J_{3,4}$= 8-8.5 Hz, $J_{3,5}$= 1.5-2.5 Hz, $J_{4,5}$= 6-8 Hz, $J_{4,6}$= 1.5-2.5 Hz, $J_{5,6}$= 4-6 Hz

[c] these assignments may also be interchanged

[d] these signals are present in a complex region between 6.4 and 6.7 ppm

[e] not observed among the phenyl signals

Table II. *^{13}C NMR data of aminopyridines and N-methylaminopyridines in $CDCl_3$ at 35°C and of their anions in liquid ammonia containing potassium amide at -50°C*[a]

Compound		C-2	C-3	C-4	C-5	C-6	CH_3	$^3J_{^{13}C\text{-}NH}$ (Hz)
4-aminopyridine	neutral[19,b]	149.6	109.8	156.3	109.8	149.6	-	
	anion 1a	148.1[c]	112.9	168.6	111.0	148.4[c]	-	5(C-3), 13(C-5)
4-amino-2,6-dimethylpyridine	neutral	158.5	106.5	153.7	106.5	158.5	24.4	
	anion 2a	154.4[c]	108.7	169.1	106.6	154.8[c]	24.0; 24.2	8(C-3). 15(C-5)
4-amino-3-methylpyridine	neutral	150.3	117.0	151.8	109.1	148.3	14.0	
	anion 3a	d	116.4	166.7	110.3	d	15.6	15(C-5)
	anion 3b	d	117.9	166.8	111.3	d	16.5	8(C-5)
4-(methylamino)pyridine	neutral	149.8	107.3	154.9	107.3	149.8	29.2	
	anion 4a	149.5	102.4	163.0	115.0	146.1	36.5	
3-amino-6-methylpyridine[12]	neutral	136.9	140.3	122.8	123.3	148.3	23.3	
	anion 6a	138.3	159.8	118.2	123.2	130.3	22.3	13(C-4)
	anion 6b	137.6	160.0	120.0	123.2	130.3	22.3	8(C-4)
3-(methylamino)pyridine	neutral	135.5	146.0	118.1	124.0	137.7	30.0	
	anion 9a	129.5	159.2	123.0	122.3	125.1	37.3	
	anion 9b	143.6	159.2	107.4	124.8	122.6	37.3	
2-aminopyridine	neutral[20,e]	160.5	108.5	136.8	111.7	147.8	-	
	anion 10a	172.7	112.9	134.9	100.4	149.5	-	6(C-3)
	anion 10b	171.2	111.2	134.7	99.9	149.0	-	13(C-3)
2-amino-5-methylpyridine	neutral[20,e]	158.3	108.4	138.0	121.0	147.4	17.1	
	anion 11a	171.5	112.6	136.3	107.4	148.2	17.2	small (C-3)
	anion 11b	170.2	110.8	136.3	106.8	147.8	17.2	13(C-3)
2-(methylamino)pyridine	neutral	160.0	106.3	137.6	112.7	148.2	29.0	
	anion 13a	166.7	114.5	132.2	99.1	149.1	35.5	
	anion 13b	168.6	102.0	136.2	100.1	149.6	36.6	
4-aminopyrimidine	neutral[21,f]	158.3	-	163.2	105.1	154.6	-	
	anion 14a	159.2	-	169.0	108.6	150.2	-	12(C-5)
	anion 14b	159.8	-	171.1	110.0	150.1	-	7(C-5)

[a] chemical shifts relative to Me_4Si (δ = 0 ppm)
[b] measured in ethanol
[c] these assignments may be interchanged
[d] a complex spectrum is found at 146.6-147.1 ppm
[e] measured in hexamethylphosphoramide
[f] measured in dimethylsulphoxide

present on the *para* ring nitrogen atom. This decreased electron density on N-3 and on the NH^- group may cause a less efficient complexation of the potassium cation[14]. As a result hydrogen bonding[13] and mutual repulsion of the electron pairs will favour 14a.

Conclusion

From this study it is evident that an electron pair is "larger" than a proton on an NH^- group, but it cannot be completely ascribed to steric factors. It is possible that the preferred isomer is also favoured by a better solvation than the other isomer and there may also exist a difference in electronic stabilization. The results are further complicated by the fact that there are two lone pairs present on the NH^- group which will both have some conjugation with the aromatic ring.
It is clear from this study that, in contrast to what has been observed with the anions of the methylamino pyridines, the carbon atom in the *syn* orientation to the lone pair appears at higher field than the corresponding carbon in the *anti* position. As the chemical shifts of the *syn* carbon atoms in the methylamino pyridines are predominantly determined by steric compression[2], it is evident that other factors work here in an opposite direction.

Experimental

The procedures followed to obtain the ^1H and ^{13}C NMR spectra have been described previously[1].
All compounds were commercially available or synthesized according to known procedures (4-amino-2,6-dimethylpyridine[15], 4-amino-3-methylpyridine[16], 5-amino-2-methylpyridine[17], 3-amino-2-methylpyridine[17], 3-amino-4-methylpyridine[17], 4-amino-6-methylpyrimidine[18] and 4-amino-6-phenylpyrimidine[18]).

Acknowledgement

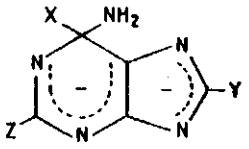
We are indebted to Dr.J.F.J.Engbersen and Dr.M.J.A.de Bie (State University of Utrecht) for helpful discussions and to Mr.M.van Dijk for his assistance.

References and Notes

1. N.J.Kos, J.Breuker, H.C.van der Plas and A.van Veldhuizen, Heterocycles, in the press.
2. N.J.Kos, J.Breuker, H.C.van der Plas and A.van Veldhuizen, J.Org.Chem. submitted.
3. F.W.Wehrli and T.Wirthlin, Interpretation of Carbon-13 NMR Spectra, Heyden and Son, London, 1978.
4. J.Almog, A.Y.Meyer and H.Shanan-Atidi, J.Chem.Soc.Perkin Trans II, 451 (1972).
5. D.Nasipuri and S.K.Konar, J.Chem.Soc.Perkin Trans II, 269 (1979).
6. D.Gust and M.W.Fagan, J.Org.Chem. 45, 2511 (1980).
7. F.G.Riddell, The conformational Analysis of Heterocyclic Compounds, Academic Press, 1980.
8. K.Brown, A.R.Katritzky and A.J.Waring, J.Chem.Soc.B, 487 (1967).
9. M.A.Robb, W.J.Haines and I.G.Csizmadia, J.Am.Chem.Soc. 95, 42 (1973).
10. It seems possible that hyperconjugation with the methyl group can lead to more stabilization in 3a (trans configuration) than in 3b.
11. Only little steric hindrance is supposed to be present in 3-methyl-4-(dimethylamino)pyridine (Z.Proba and K.L. Wierzchowski, J.Chem.Soc.Perkin Trans II, 1119 (1978)).
12. For uniformity 7 is considered as 3-amino-6-methylpyridine instead of 5-amino-2-methylpyridine.
13. G.Dodin, M.Dreyfus and J.E.Dubois, J.Chem.Soc.Perkin II, 438 (1979).
14. R.G.Shepherd and J.L.Fedrick, Reactivity of Azine, Benzoazine and Azinoazine Derivatives with Simples Nucleophiles in Advances of Heterocyclic Chemistry, vol.4, A.R.Katritzky Ed., Academic Press, New York, 1965.
15. J.Suszko and M.Szafran, Roczn.Chem. 39, 709 (1965); through C.A. 64, 3466 h (1966).
16. E.C.Taylor and A.J.Crovetti, J.Org.Chem. 19, 1633 (1954).
17. L.van der Does and H.J.den Hertog, Recl.Trav.Chim.Pays-Bas 84, 951 (1965).
18. H.C.van der Plas, Recl.Trav.Chim.Pays-Bas 84, 1101 (1965).
19. H.L.Retcofsky and R.A.Friedel, J.Phys.Chem. 71, 3592 (1967).
20. K.Konishi and K.Takahashi, Bull.Chem.Soc.Japan 50, 2512 (1977).
21. J.Riand, M.T.Chenon and N.Lumbroso-Bader, Tetrahedron Lett. 3123 (1974).

9 DISCUSSION

The first part of this thesis describes the reactions of purine and several 2,6,- and 8-substituted purines with potassium amide in liquid ammonia. These compounds are present as anions in this strongly basic medium, due to deprotonation of the NH group of the imidazole ring. The introduction has shown that attack of a nucleophile on an anionic purine preferably takes place at position 6. The results presented in this thesis emphasize that position 6 is the most reactive for attack of the strongly nucleophilic amide ion. It has been shown that, if this position is occupied as in 6-methylpurine, 6,8-di-*tert*-butylpurine, 6-*tert*-butyl-8-(methylthio)purine, adenine, 8-chloroadenine, 2-chloro-6-methylpurine and 2-chloro-6,8-di-*tert*-butylpurine no reaction at position 2 or 8 occurs. The blocking effect of the *tert*butyl group is certainly due to its bulkiness and that of the methyl and amino group to their ability to undergo deprotonation in this strongly basic medium. Attack of the nucleophile is then prevented in the dianion thus formed. When a phenyl group occupies position 6 no blocking effect is observed. ^{15}N labeling has proved that amination of 2-chloro-6-phenylpurine into 2-amino-6-phenylpurine takes place by an initial attack of the amide ion at position 6 (Scheme I, X = C_6H_5, Y = H, Z = Cl). These results exemplify the general picture that the reaction of an anionic purine with the amide ion always starts with attack at position 6 to give a σ-adduct (Scheme I), but that the subsequent reaction sequence depends on the position of the leaving group.



In fact, three different types of reactions can be discussed.

a) *Direct substitution*

If a leaving group is present at position 6 (X = Cl, SCH_3) the σ-adduct aromatizes rapidly by expulsion of this group (S_N(AE)-mechanisme). In these cases the intermediate adduct cannot be observed. In the absence of a leaving group at position 6 a Chichibabin amination occurs, due to loss of a hydride ion

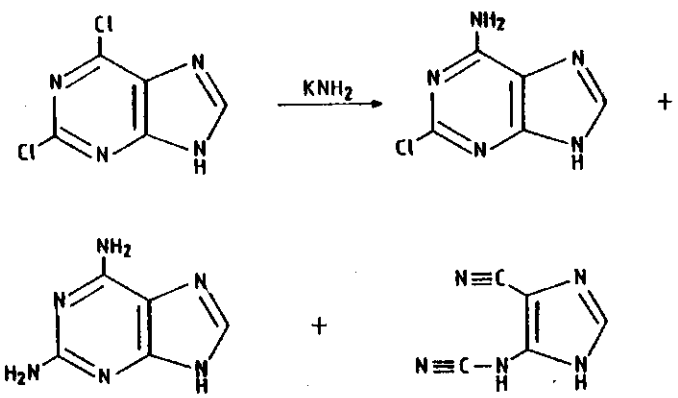
from this position. The second step in the reaction, i.e. the aromatization, is found to be rate-determining in this case since the intermediate σ-adduct can be observed by low temperature NMR spectroscopy. It is interesting that the Chichibabin amination of purine occurs only in liquid ammonia and not in other solvents, while several other heterocycles (e.g. pyridine) react better in other solvents.[1] When a leaving group is present at position 8 (Y = Cl, SCH_3) the Chichibabin amination can also occur. Thus 8-(methylthio)purine gives 8-(methylthio)adenine as sole product and 8-chloropurine likewise yields 8-chloroadenine.

b) *Tele-substitution*

An example is the formation of adenine besides 8-chloroadenine from 8-chloropurine. This tele-amination is in fact the first example of this reaction in purine chemistry. The adduct (X = Z = H, Y = Cl) protonates at position 8 and thereafter elimination occurs.

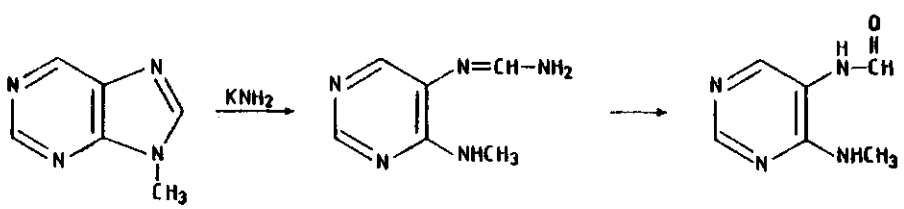
c) *Ring-Opening*

A third mechanism is operative when a leaving group is present at position 2 (Z = Cl, F, SCH_3). In this case the σ-adduct undergoes opening of the pyrimidine ring with expulsion of the leaving group. The resulting imidazole derivative undergoes ring closure on neutralization to give a 2-aminopurine. This type of reaction is referred to as an S_N(ANRORC) mechanism. It is interesting to compare these results with those of the amination of 2,6-dichloropurine. When 2,6-dichloropurine is reacted with potassium amide in liquid ammonia three products are isolated: 2-chloroadenine, 2,6-diaminopurine and 4-cyano-5-cyanoaminoimidazole (scheme II).[2] The last two products originate from 2-chloroadenine as shown by their formation when 2-chloroadenine is reacted under identical conditions.[2]



When 2-amino-6-chloropurine is treated with potassium amide in liquid ammonia only 4-cyano-5-cyanoaminoimidazole[2] is formed and no 2,6-diaminopurine. Direct attack of the amide ion at position 2 of 2-chloroadenine to give 2,6-diaminopurine is unlikely in view of the results obtained with other purines, as 2-chloro-6-phenylpurine and 2-chloro-6,8-di-*tert*-butylpurine. An S_N(ANRORC) mechanism, starting with attack at position 6 therefore seems to be involved. Adduct formation at a position already occupied by an amino group is not unprecedented. The conversion of 4-amino-2-bromoquinoline into 4-amino-2-methylquinazoline involves an initial addition at position 4.[3]

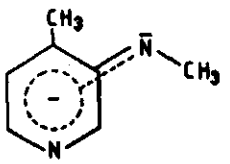
All reactions discussed above involved the anions of purines, since these are deprotonated under these strongly basic conditions. In 9-substituted purines, where anion formation is impossible, the reactivity increases, especially at position 8. The increase in reactivity can be shown by comparison of the reaction times. Both 6-chloro-9-methylpurine and 9-methylpurine react with potassium amide in liquid ammnia in about 1 h at ~80°C,[4] while anionic purines require 20 to 70 h at -30°C. 9-Methylpurine undergoes addition at position 8, followed by opening of the imidazole ring to give a substituted pyrimidine. Hydrolysis during work up gives 4-(methylamino)-5-formamidopyrimidine.[4]



6-Chloro-9-methylpurine reacts similarly, but 9-methyladenine is also formed.[4] Due to the rapid reaction it is impossible to measure NMR spectra of intermediates, but it is clear from the results that positions 6 and 8 are both attacked by the amide ion. We have observed for 8-methylthio-9-methylpurine that an adduct is initially formed at position 6, but that the products must be formed via an adduct at position 8.[4] The latter however could unfortunately not be observed.

During the ^{1}H NMR study of the reactions of 6-chloro- and 6-methylthiopurine with potassium amide in liquid ammonia, we observed geometrical isomerism in the adenine formed (Chapter 3). An unexpected result, since in this strongly

basic medium rapid exchange leading to isomerization should be expected. A detailed study of the phenomenon of geometrical isomerism in a number of anions of amino(aza)aromatics has shown that the rotational barrier in the anions of the amino compounds is higher than in the neutral molecules. This is due to delocalization of the negative change to the aromatic ring. The anion of 4-methylaniline, for instance, shows a coalescence temperature of about -30°C, whereas N-methylaniline has a coalescence temperature of about -120°C.[5,6] The assignment of the ^{1}H and ^{13}C NMR spectra to the *syn* and *anti* isomers of the anions of aminopyridines, aminopyrimidines and N-methylaminopyridines was possible by applying three criteria: *i*) In *ortho* methyl substituted-(methylamino)pyrimidines the only existing conformation is the isomer in which the two methyl groups are in the *anti* position.



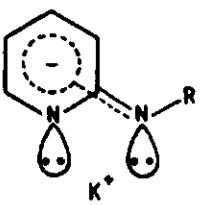
ii) The coupling constant $^{3}J_{^{13}C\text{-}NH}$ between the proton of the NH^- group and the *ortho* carbon atoms is larger for the conformation in which the hydrogen atom of the NH^- group is in the anti position relative to the *ortho* carbon atom than for the *syn* conformation.

iii) In the anions of the methylaminopyridines and the amino compounds the *ortho* hydrogen atom in the *syn* position relative to the lone pair resonates at a lower field than the hydrogen atom in the *anti* position.

Electric field effects are important in determining the chemical shift of hydrogen atoms. It is therefore not surprising that this relationship holds for both NH^- and NCH_3^-.

However, the ^{13}C NMR chemical shifts for the ortho carbon atoms are not comparable in the anions of amino- and methylaminopyridines. In methylaminopyridine the *ortho* carbon atom in the *syn* position relative to the lone pair resonates at a lower field than the *ortho* carbon atom in the *anti* position. In the anions of aminopyridines this criterion is reversed. These rules were proven by comparison with reference compounds and selective decoupling. This can be explained by the influence of the methyl group resulting in a large upfield shift of the *ortho* carbon atom in the syn position relative to the lone pair.[7]

This chemical shift arises from steric perturbation of the carbon nucleus and is known as steric compression shift. The assignment of the ^{1}H and ^{13}C NMR spectra of the aminopyridines and their *ortho* methyl derivatives to the *syn* and *anti* isomers has shown that the *ortho* methyl substituent causes a preference for the isomer in which the hydrogen atom of the NH^{-} group and the methyl group are in the *anti* orientation. This has been described in terms of the lone pair being "larger" than a proton, but it must be emphasized that it is possible that the preferred isomer is also favoured by better solvation and by an extra electronical stabilization.
It has also been shown that in 2-aminopyridine, 2-methylaminopyridine and 4-aminopyrimidine the conformation with the electron pairs in the *syn* position is stabilized by complexation with the potassium cation. Assignment of the ^{1}H NMR spectrum of aminopyrazine (Chapter 6) also supports this conclusion.



References

(1) A.F.Pozharskii, A.M.Simonov and V.D.Doron'kin, Russ. Chem. Rev., 47, 1042 (1978).
(2) N.J.Kos and H.C.van der Plas, unpublished results.
(3) H.J.den Hertog and D.J.Buurman, Recl. Trav. Chim. Pays-Bas, 91, 841 (1972).
(4) N.J.Kos, H.C.van der Plas and W.J.F.Blees, unpublished results.
(5) L.Lunazzi, C.Magagnoli, M.Geurra and D.Macciantelli, Tetrahedron Letters, 3031 (1979).
(6) F.A.L.Anet and M.Ghiaci, J. Am. Chem. Soc., 101, 6857 (1979).
(7) C.A.Bunnell and P.L.Fuchs, J. Org. Chem., 42, 2614 (1977).

SUMMARY

In this thesis two subjects are described: a. the amination of substituted purines by potassium amide in liquid ammonia and b. the occurrence of geometrical isomerism in the anions of aromatic amino compounds.

It is shown that the first step in the amination of purines, being present as anions under these strongly basic conditions, is the formation of a σ-adduct as position 6 to give a 6-amino-1,6-dihydropurinide. If position 6 is occupied by a blocking group an attack at position 2 or 8 does not occur. The further reaction course depends on the nature of the substituents and their position in the purine ring. i. If a leaving group (Cl,SCH_3) is present at the same position where the amide ion has attacked, this substituent is expelled ($S_N(AE)$ mechanism). In case no leaving group is present a Chichibabin amination occurs due to expulsion of a hydride ion from position 6 (this reaction is described in Chapter 2).

The Chichibabin amination can also occur at position 6 when a leaving group (Cl,SCH_3) is present at position 8. ii. In the last-mentioned system a *tele* substitution is possible besides the $S_N(AE)$ reaction. This reaction is exemplified in the conversion of 8-chloropurine into adenine (formed besides 8-chloroadenine). The σ-adduct at position 6 is protonated at position 8, after which dehydrohalogenation occurs ($S_N(AE)^{tele}$, see Chapter 3). iii. If a leaving group is present at position 2 (Cl,F,SCH_3) the σ-adduct at position 6 undergoes ring opening of the pyrimidine ring with expulsion of the leaving group. The resulting imidazole derivative undergoes ring closure to give a 2-aminopurine. This type of reaction is referred to as an $S_N(ANRORC)$ mechanism and is described in Chapter 4.

It has been established that in an $S_N(AE)$ mechanism the second step, involving the expulsion of the leaving group, is fast; the intermediary σ-adduct cannot be observed. However, in the Chichibabin amination, *tele* amination and reaction according to the $S_N(ANRORC)$ mechanism, the second step is slow and therefore the σ-adduct can be observed by low temperature NMR spectroscopy.

In Chapter 5 a new method is presented for the reductive removal of amino and alkylamino groups from position 6 of 9-substituted purines with sodium in

liquid ammonia. The reaction involves reduction of the N(1) - C(6) bond, followed by elimination. This reaction is of special interest since the alternative method for the removal of amino groups i.e. the diazotization cannot be used with alkylamino groups. Therefore this new method is especially useful for the deamination of 6-(alkylamino)-9-substituted purines.

In the last part of this thesis the occurrence of geometrical isomerism in the anions of aromatic amino compounds in liquid ammonia containing potassium amide is described. It is shown that this phenomenon occurs even in anilines, where the rotational barrier will be lower than in azaaromatic systems. This is confirmed by the occurrence of coalescence with increasing temperature (Chapter 6). The ^{1}H and ^{13}C NMR spectra of the anions of aminopyridines, aminopyrimidines and N-methylaminopyridines are assigned to the *syn*- and *anti* isomers. It has been revealed that in all these anions the *ortho* hydrogen atom in the *syn* position relative to the lone pair resonates at a lower field than the hydrogen atom in the *anti* position. For the ^{13}C NMR shifts of the *ortho* carbon atoms it was found that in the anions of N-methylaminopyridines the *ortho* carbon atom in the *syn* position relative to the lone pair resonates at lower field than the *ortho* carbon atom in the *anti* position.

In the anions of aminopyridines and aminopyrimidines this phenomenon is reversed. We have also shown that the presence of a methyl group *ortho* to the anionic amino group causes a preference for the isomer, in which the proton of the NH^- group is in a *syn* position relative to the methyl group. This is explained in terms of the electron pair being "larger" than a proton, but it is possible that the preferred isomer is also stabilized by a better solvation and by an electronical effect.

SAMENVATTING

Dit proefschrift behandelt twee onderwerpen: a. de aminering van gesubstitueerde purinen met kaliumamide in vloeibare ammoniak en b. het optreden van geometrische isomerie in de anionen van aromatische aminen.

Er wordt aangetoond dat in de eerste stap in de aminering van purinen, die onder de sterk basische omstandigheden als anionen aanwezig zijn, de vorming van een σ-adduct op plaats 6 optreedt. Hierbij ontstaat het anion van een 6-amino-1,6-dihydropurine. Als de 6-plaats geblokkeerd is, treedt geen aanval op plaats 2 of 8 op.

Het verdere verloop van de reactie hangt af van de aard en positie van de substituenten. i. Als er een vertrekkende groep (Cl, SCH_3) aanwezig is op dezelfde plaats waar zich de aminogroep bevindt, wordt deze substituent afgesplitst. ($S_N(AE)$ mechanisme). Als er op deze plaats geen substituent (Cl of SCH_3) aanwezig is - dus alleen een H-atoom - treedt er vorming van de aminoverbinding op (Chichibabin aminering), waarbij de waterstof als hydride ion wordt afgesplitst (hoofdstuk 2). Deze reactie vindt ook plaats indien er een vertrekkende groep (Cl,SCH_3) aanwezig is op plaats 8. Uit 8-chloor- of 8-(methylthio) purine ontstaat dan 8-chloor- of 8-(methylthio)adenine. ii. Het is gebleken dat 8-chloorpurine bij behandeling met kaliumamide ook een z.g. *tele*-substitutie kan ondergaan. Hierbij ontstaat uit 8-chloorpurine adenine (naast 8-chlooradenine). Het σ-adduct op plaats 6 wordt geprotoneerd op plaats 8, waarna dehydrohalogenering optreedt ($S_N(AE)^{tele}$, hoofdstuk 3). iii. Als er een vertrekkende groep aanwezig is op plaats 2 (Cl,F,SCH_3) ondergaat het σ-adduct een opening van de pyrimidinering. Het ontstane imidazoolderivaat ondergaat ringsluiting tot een 2-aminopurine. Dit type reactie wordt aangeduid als een S_N(ANRORS) mechanisme en wordt beschreven in hoofdstuk 4. Er is vastgesteld dat in een $S_N(AE)$ reactie de tweede stap, *d.w.z.* de afsplitsing van de vertrekkende groep, snel verloopt; het σ-adduct kan dus niet als intermediair worden waargenomen. In de Chichibabin aminering, de *tele*-aminering en de reacties, die verlopen volgens het S_N(ANRORS) mechanisme, is de tweede stap langzaam; het σ-adduct kan nu bij lage temperatuur worden waargenomen met behulp van NMR spectroscopie.

In hoofdstuk 5 wordt een nieuwe methode beschreven voor het reductief afsplitsen van amino- en alkylaminogroepen van plaats 6 in 9-gesubstitueerde purinen met behulp van natrium in vloeibare ammoniak. De reactie verloopt *via* reductie van de N(1)- C(6) band, gevolgd door eliminering. Deze methode is interessant daar de alternatieve methode voor de afsplitsing van aminogroepen *via* diazotering onbruikbaar is voor verwijdering van alkylaminogroepen.
In het laatste deel van dit proefschrift wordt het optreden van geometrische isomerie in de anionen van aromatische aminen in aanwezigheid van kaliumamide in vloeibare ammoniak beschreven. Er wordt aangetoond dat geometrische isomerie zelfs in anilines, waar de rotatiebarrière lager zal zijn dan in azaaromaten, optreedt. Dit wordt bevestigd door het optreden van coalescentie bij toenemende temperatuur (hoofdstuk 6).
Het is mogelijk gebleken de ^{1}H en ^{13}C NMR spectra te interpreteren op basis van toekenning aan de *syn* en *anti* isomeren van de anionen van aminopyridinen, aminopyrimidinen en N-methylaminopyridinen. Het *ortho* waterstofatoom in de *syn*-positie ten opzichte van het vrije elektronenpaar blijkt in al deze anionen bij lager veld te resoneren dan het waterstofatoom in de *anti*-positie. Voor de ^{13}C NMR verschuivingen van de *ortho* koolstofatomen wordt gevonden dat in de anionen van N-methylaminopyridinen het *ortho* koolstofatoom in de *syn*-positie ten opzichte van het vrije elektronenpaar ook bij lager veld resoneert dan het *ortho* koolstofatoom in de *anti*-positie, maar dat dit in de anionen van aminopyridinen en aminopyrimidinen precies omgekeerd is. We hebben verder aangetoond dat de aanwezigheid van een methylgroep op de *ortho* plaats ten opzichte van de NH^- groep een voorkeur veroorzaakt voor het isomeer, waarin het proton van de NH^- groep *syn* staat ten opzichte van de methylgroep. Dit wordt verklaard door aan te nemen dat het elektronenpaar "groter" is dan een proton, maar het wordt niet onmogelijk geacht dat het voorkeursisomeer ook gestabiliseerd wordt door een betere solvatatie en door een elektronisch effect.

CURRICULUM VITAE

Na het behalen van het diploma gymnasium β aan het Rembrandt Lyceum te Leiden in 1969 werd in september van dat jaar begonnen met de studie in de scheikunde aan de Rijksuniversiteit te Leiden.
Het kandidaatsexamen (richting S 2) werd in juni 1972 afgelegd. De studie werd voortgezet onder leiding van Prof.Dr.E. Havinga (organische chemie), Prof.Dr.V. Ponec (heterogene katalyse) en Dr.F.C. Mijlhoff (elektronendiffractie). Het doctoraalexamen werd in oktober 1975 cum laude afgelegd.
Sinds november 1975 ben ik als wetenschappelijk medewerker werkzaam op het Laboratorium voor Organische Chemie van de Landbouwhogeschool te Wageningen, aanvankelijk in dienst van de Nederlandse Organisatie voor Zuiver Wetenschappelijk Onderzoek (Z.W.O.), vanaf november 1979 in dienst van de Landbouwhogeschool. Aldaar werd het in dit proefschrift beschreven onderzoek verricht. Daarnaast ben ik werkzaam geweest op de tweede- en derdejaars practica.