SYNTHESIS AND FLUORESCENCE CHARACTERISTIC OF 2-SUBSTITUTED AND 6-SUBSTITUTED PURINES

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Abstract

2-Fluoropurine was prepared from 2–aminopurine through a diazotization reaction, followed by treatment with fluoroboric acid. 2-Aminopurine was obtained from a series of reactions, using 5-nitrouracil as the starting material. 2-Piperidino and 2-anilinopurines were obtained by treating 2-fluoropurine with piperidine and aniline respectively. 6-Piperidino and 6-anilinopurines were obtained by treating 6-chloropurine with piperidine and aniline. Fluorescence studies was carried out in various solvents and maximum fluorescence was observed in 75% ethanol. 2-Aminopurine showed the highest fluorescence intensity, followed by 2-anilino and 2-piperidinopurines. 6-Substituted purines showed stronger fluorescence intensities compared to 2-substituted purines.

Abstrak

2-Fluoropurina disediakan daripada 2-aminopurina melalui tindak balas pendiazoan diikuti dengan pengolahan dengan asid fluoroborik. 2-Aminopurina diperolehi melalui beberapa peringkat tindak balas bermula dengan 5-nitrourasil. 2-Piperino dan 2-anilinopurina diperolehi apabila 2-fluoropurina ditindak balas dengan piperidina dan anilina. 6-Anilino dan 6-piperidinopurina pula diperolehi apabila 6-kloropurina ditindak balaskan dengan piperidina dan anilina. Kajian pendarfluor dilakukan menggunakan berbagai pelarut dan kadar pendarfluor maksimum diperolehi dalam 75% etanol. 2-Aminopurina menunjukkan kadar pendafluor yang tinggi diikuti dengan 2-anilino dan 2-piperidino purina. Purina tertukar ganti di kedudukan 6 menunjukkan kadar pendafluor yang lebih tinggi berbanding dengan purina tertukar ganti di kedudukan ke 2 yang setara.

Introduction

The purine system is one of the most important systems present in living systems. The purine system or rings can be found in many natural products, including nucleotides, co-enzymes and several compounds which are valuable for treatment of cancer. Even though many of its rings can be found in the natural occurring products, the parent compound itself cannot be found in nature.

The first pure purine, uric acid, was obtained by the isolation from kidneystones in 1776 [1] but its structure was confirmed one hundred year later by Medicus [2]. But the chemistry of purines flourished from 1906 onwards [3-5] until today.

The purine ring can be synthesized using two main synthetic route, either using pyrimidine or imidazole as the precursor. The most extensively used method is the Traube Sythesis which involved the use of diaminopyrimidines, whereby the diaminopyrimidines are condensed with a simple compound to supply a one-carbon fragment to bridge the two pyrimidine amino nitrogen atoms to form a five-membered ring. In this work, the Traube method was used in the synthesis of 2-fluoropurine.

The fluorescence characteristic of purines or other heterocycles are not extensively studied, eventhough a wide variety of heterocyclic compounds are known to be fluorescent [6]. Fluorescence studies of these compounds are made more difficult because their fluorescence characteristics are often dependent on the solvents used. The aim of this work is to study the fluorescence charateristic of selected alkylaminopurines and the effect of solvent on the fluorescence characteristic. In this paper, only the characteristic of 2- and 6- anilino, 2- and 6-piperidino purines will be discussed.

Experimental

Synthesis of 2-fluoropurine

2,4-Dichloro-5-nitropyrimidine [7]

5-Nitrouracil (26 g), phosphoryl chloride (130 ml), and dimethylaniline (32 ml) were heated with occasional shaking until the reaction commerced. When this has subsided, the mixture was refluxed for 1.5 hours, cooled and phosphoryl chloride was evaporated off. The residue was poured onto crushed ice with vigorous stirring, and extracted with ether (500 ml). The ether extracts were washed with water and dried over anhydrous sodium sulphate. Removal of ether and vacuum distillation gave pure product.

89%, IR (cm^{-1}):1670, 1620, 1350, 785; ^{1}H NMR ($CDCl_3$) δ:9.26, s, 1H, (H_6).

2, 4-Diamino-5-nitropyrimidine [8]

2, 4-Dichloro-5-nitropyrimidine (10.3 g) and phenol were heated under reflux while a stream of ammonia was passed into the mixture for four hours. The phenol was distilled off and the residual suspension was cooled and filtered. The crystals of 2, 4-diamino-5-nitropyrimidine were washed with water, followed by ethanol and dried.

90%, IR (cm^{-1}): 3345, 1675, 1630, 1350; ^{1}H NMR ($CDCl_3$) δ: 9.15, s, 1H, (H_6), 7.20, b, 2H (NH_2 of C_4), 6.50, b, 2H (NH_2 on C_2).

2, 4, 5-Triaminopyrimidine [8]

Finely powdered 2, 4-diamino-5-nitropyrimidine (8.9 g) was heated to 80 ^{o}C with water (15 ml) and mechanically stirred. Sodium dithionite (3.75 g) was added during 3 - 4 minutes. The resulting solution was stirred until 60 ^{o}C, followed by addition of powdered anhydrous sodium carbonate (5.5 g). The thick suspension was taken to dryness in an open basin on water bath. The solid was machine ground and extracted for 20 minutes with stirred boiling alcohol (22 ml) which was filtered while hot. The filtrate was taken to dryness and the product was re-extracted with ethanol (7.5 ml) to remove sodium carbonate. Evaporation of filtrate gave the pure product.

71%; IR (cm^{-1}): 3363, 1670, 1625; ^{1}H NMR (DMSO-d6) δ: 7.25 s, 1H (H_6), 6.00, b, 4H (NH_2 of C_2 and C_4), 5.10, b, 2H (NH_2 of C_5).

2-Aminopurine [9]

2, 4, 5-Triaminopyrimidine (0.3 g), formyl morpholine (1.2 ml) and formic acid (0.6 ml) was heated under refluxed for one hour in nitrogen atmosphere. Acetone (2 ml) was added to precipitate 2-aminopurine which was dissolved in boiling water (3 ml). The solution was passed through a wide filter, cooled to 50 ^{o}C and diluted with 3N nitric acid and refrigerated. The nitrate was filtered off. The solution was then suspended in boiling water and brought to pH 6.8 with sodium citrate and 6N sodium hydroxide solution. The solution was boiled with charcoal, giving buff-coloured crystals of 2-aminopurines. Crystallisation with boiling water gave colourless crystals.

40%, IR (cm^{-1}): 3245, 1670, 1620; ^{1}H NMR (D_2O) δ: 8.70, s, 1H (H_6), 8.00 s, 1H (H_8) 6.25, b, 2H (NH_2), 6.50, b, 1H (NH).

2-Fluoropurine (improved method) [10, 11]

An aqueous solution of sodium nitrite (800 mg in 4 ml of water) was added with stirring to a solution of 2-aminopurine (200 mg) in 48% fluoroboric acid (22 ml) at the rate of 0.1 ml per minute at -11 ^{o}C. After the addition was completed, the mixture was stirred for one hour between - 10 to 0 ^{o}C. The mixture was then neutralized with 50% sodium hydroxide solution. The neutral slurry was evaporated to dryness. The crude purine was isolated by extraction of dry residue with ether in a soxhlet extractor. Evaporation of ether gave crude product, which was recrystallised from water.

35%, IR (cm^{-1}): 3325, 1673, 1623, 1132; ^{1}H NMR (DMSO-d6) δ:8.70 s, 1H, (H_6), 8.00 s, 1H (H_8), 6.50, b, 1H (NH).

2-Piperidinopurine

Piperidine (20 mg) in ethanol (4 ml) was added to a solution of 2-fluoropurine (40 mg) in ethanol (7 ml) and the mixture was refluxed for 1 hour at 100 ^{o}C. The mixture was cooled and ethanol was evaporated off. The slurry was extracted twice with ether. The ethereal layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave crude product, which was recystallised from petroleum ether.

50%, decomposed above 215, IR (cm^{-1}): 3115, 1673, 1620; ^{1}H NMR δ: 8.70, s, 1H (H_6), 8.00, s, 1H (H_1), 3.35, m, 4H ($H_{2'}$, $H_{6'}$), 1.68, m, 6H ($H_{3'}$, $H_{4'}$, $H_{5'}$); M^+: 203.1163.

2-Anilinopurine

2-Fluoropurine (80 mg) was added to aniline (3 ml) and warmed at 60 °C for four hours. The mixture was cooled and refrigerated overnight. 2-Anilinopurine was crystallized out of the reaction mixture. The crystal was filtered, washed with ice-cold water and dried. Pure product was obtained after recrystallisation from dichloromethane.

73.5%, decomposed above 200°, IR (cm^{-1}): 3330, 1670, 1630; ^{1}H NMR (DMSO-d6) δ: 8.65, s, 1H (H_6), 8.00, s, 1H (H_8), 7.20, m, 3H ($H_{3'}$, $H_{4'}$, $H_{5'}$), 6.90, d, 2H ($H_{2'}$ and $H_{6'}$), 5.40, d, 2H, (N-H); M^+:211.0858.

6-Piperidinopurine

Piperidine (0.5 ml) in ethanol (3 ml) was added to 6-chloropurine (0.232 g) [11-12] and the mixture was refluxed for 2 hours. The mixture was cooled and ethanol was evaporated off. The slurry was extracted twice with ether; the ethereal layer was washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave grey product, which was recystallised from petroleum ether.

30%, decomposed above 270, IR (cm^{-1}): 3115, 1673, 1620; ^{1}H NMR (DMSO-d6) δ: 8.23, s, 1H (H_2), 8.15, s, 1H (H_8), 2.62, m, 4H ($H_{2'}$, $H_{6'}$), 1.66, m, 6H ($H_{3'}$, $H_{4'}$, $H_{5'}$); M^+: 203.1163.

6-Anilinopurine

2-Chloropurine (0.161 g) was added to aniline (0.3 ml) in ethanol (3 ml) and refluxed for 3 hours. The mixture was cooled and the solvent was evaporated. The slurry was extracted with ether, washed with water and dried over anhydrous sodium sulphate. Evaporation of ether gave light brown solid.

50.3%, decomposed above 280°, IR (cm^{-1}): 3330, 1670, 1630; ^{1}H NMR (DMSO-d6) δ: 9.81, s, 2H (N-H), 8.43, s, 1H (H_2), 8.34, s, 1H (H_8), 8.01, d, 2H ($H_{2'}$ and $H_{6'}$), 7.38, t, 2H ($H_{3'}$, $H_{5'}$), 7.08, t, 1H ($H_{4'}$); M^+: 211.0858.

Spectroscopic Analysis

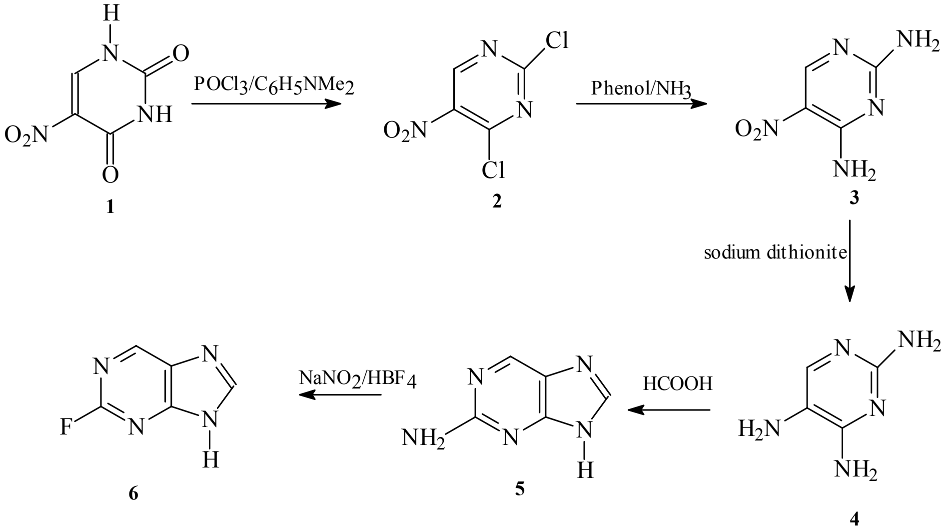
All solvents were redistilled before use. Melting points were determined with Electrothermal Melting Point Appararus and were not corrected. Infrared spectra were recorded using Perkin Elmer 298 Infrared Spectrometer and FTIR Perkin Elmer 1600 Series. ^{1} H NMR spectra were recorded on Bruker WP-80 and Bruker AM 250.

Fluorescence Studies

2- and 6-Substutitued purines at the same concentration were prepared in various solvents. Quinine sulphate with the same concentration as the compounds under studied were also prepared and used as the standard. The fluorescence intensity of quinine sulphate was taken to be 1.00. The fluorescence measurement was carried out using Hitachi Fluorescence Spectrometer Model F-2000.

Results and Discussion

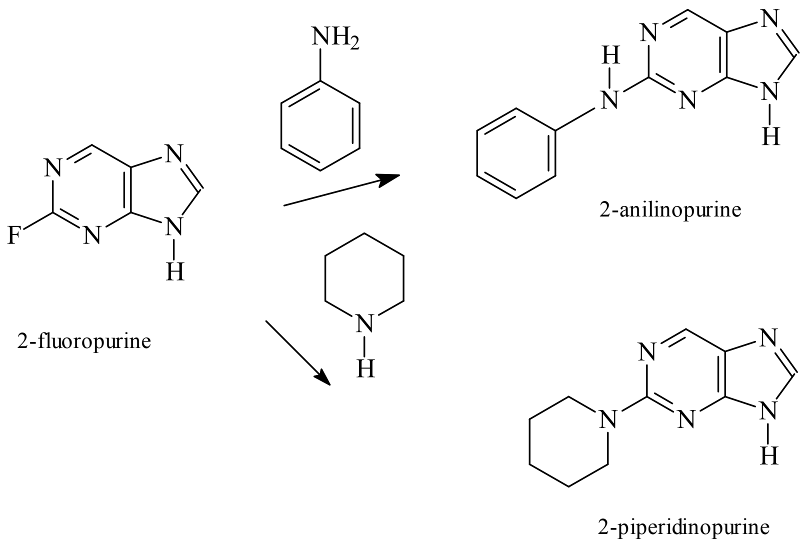
The synthetic route of 2-fluoropurine is as shown in Scheme 1 below:-



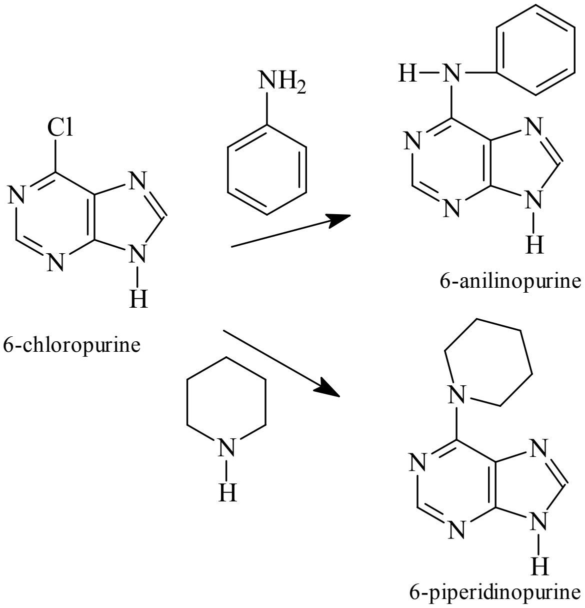
Scheme 1

The preparation of 2-fluoropurine was carried out according to the Brown method [4], using 5-nitrouracil (1) as the starting material. Purine ring was obtained through cyclisation reaction of 2, 4, 5-triaminopyrimidine (4) with formic acid to give 2-aminopurine (5). Compound 5 undergoes diazotization reaction, followed by treatment with HBF_4 to give 2-fluoropurine (6). Low percentage yield was obtained in the synthesis of 6-chloropurine. Due to low percentage yield obtained, 6-chloropurine used in this work was obtained commercially.

Treatment of 2-fluoropurine and 6-chloropurine with aniline and piperidine are as shown in Schemes 2 and 3.



Scheme 2



Scheme 3

The structures of 2- and 6- substituted purines were confirmed by ^1H NMR and mass spectra as given in the experimental section.

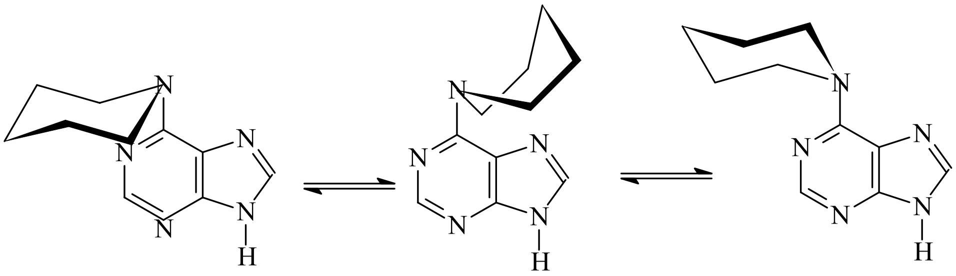
The fluorescence studies of 2- and 6-substituted purines were carried out in 75% ethanol. Quinine sulphate at the same concentration as the compounds studied was used as the standard and its fluorescence intensity was taken to be 1.00. The fluorescence band of 2- and 6-substituted purines were given in Table 1.

Table 1: Fluorescence peaks of 2- and 6- substituted purines in 75% ethanol

Purine	Solvent	Excitation wavelength/nm	Fluoresence wavelength/nm	Rel. Fluorescence intensity
2-fluoro	75% EtOH	350	430	0.640
2-amino	75% EtOH	340	380	0.964
2-anilino	75% EtOH	340	473	0.371
2-piperidino	75% EtOH	340	390	0.307
6-chloro	75% EtOH	330	420	0.650
6-anilino	75% EtOH	340	480	0.452
6-piperidino	75% EtOH	340	395	0.351

It can be seen from the table that, the fluorescence intensity of 6-substituted purines is stronger than 2-substituted derivatives. This is probably due to at the 6- position, the substituent is not sandwich between the two nitrogen atoms as in the 2nd position. As the result, the electrons can move freely from the substituent to the rest of the ring.

Anilinopurines showed stronger fluorescence intensities and fluoresced at a higher wavelength compared to the piperidinopurines. The fluorescence peak observed at a higher wave length is believed to be due to the increase in the degree of conjugation in the anilinopurines compared to piperidinopurines. The same phenomena was observed with pyrimidine derivatives studied earlier [13]. Lower fluorescence intensity was recorded with piperidinopurines in 75% ethanol. This is probably due to the piperidino ring flipping from one conformation to another, resulted in the loss of energy in the transition state. As the result, low fluorescence was observed.



Flipping of piperidino ring

2- and 6-Piperidino purines were less rigid compared to 2- and 6- anilino purines. Some energy may also loss in the transition state, which also resulted in low fluorescence intensity observed.

2-Aminopurines showed the highest fluorescence intensity amongst all the derivatives of purines studied. This is probably due to the electron donating nature of the amino group which enhances the mobility of electron in the system. The free mobility of the electron enhances the □to □ transitions, which resulted in high fluorescence intensity.

Conclusion

All the 2- and 6-substituted purines studied are fluorescent compounds. 2-Aminopurine is the most fluorescent and the fluorescence intensity depending on the type of the substituents. Substituent with high degree of conjugation, as in the case of anilinopurine showed higher fluorescence intensity and fluoresced at a higher wavelength. The purine system with unconjugated substituent (in the case of piperidinopurines) fluoresced at a lower wavelength. Further work on the purine system and other heterocyclic systems are still under going before any concrete conclusion can be made on solvent-structure-fluorescence and substituent-fluorescence relationship.

Acknowledgement

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