THE SYNTHESIS AND POLYMERIZATION OF A VINYL DERIVATIVE OF 6-METHYLTHIOPURINE

by

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ABSTRACT

THE SYNTHESIS OF VINYL DERIVATIVE OF 6-MERCAPTOPURINE AND ITS POLYMERIZATION

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The present work pertains to the modification of 6-mercaptopurine, a drug used in cancer therapy. The drug is highly toxic and non-specific towards abnormal cells. It is also absorbed by the normal cell and interferes in the biosynthesis. To overcome these inherent draw backs, attempts were made to modify 6-mercaptopurine so that one or more of the following characteristics are achieved: a) reduced toxicity, b) greater specificity, c) slow release of the drug into the system being treated.

The therapeutic value of the drug is associated with the reactivity of the 6-thio group. The 9-position offers the best site for the modification, since substitution at any other position, in most cases, reduces the anti-tumor activity of the drug. The general aim is to introduce at position-9 a side chain with a vinyl group so that the polymerization could be attempted through the unsaturated group.

Previous workers have introduced acryloyl and allylcarbamate groups at the 9-position with a hope to polymerize the monomer and thus obtain the desired objectives. These studies were only partially successful. The first monomer could not be isolated in pure form and the second pure monomer failed to polymerize, probably because of its allylic nature. With the failure of these two attempts, the next logical approach was to try a vinyl-carbamate group. The present work is an attempt in this direction.

6-Mercaptopurine was first converted to 6-methylthiopurine to ensure the modification at the 9-position. Next the 6-methylthiopurine was reacted with vinyl isocyanate in anhydrous benzene in the presence of triethylamine. Hydroquinone was incorporated in the reaction system to prevent homopolymerization of vinyl isocyanate and/or the resulting monomer.

The preparation of the compound posed no serious problem. The most difficult part was purification and it consumed the major portion of the thesis work. The initial attempts were to recrystallize from a chloroform/hexane mixture as reported in the literature for related compounds. About a dozen mixtures of solvents and non-solvents were tried with no success. A sample obtained after thirteen successive recrystallizations gave elemental values close to the theoretical expectations. Relying on melting point as a criteria

of purity turned out to be misleading as the compound decomposed just at the melting point. The IR-spectrum showed a C=C bond and the three amide peaks at the probable regions. Further continuation of recrystallization did not improve the degree of purity, therefore, resorted to a chromatographic technique. Elution of the monomer from a silica gel column using chloroform was very successful and is the most efficient way of purifying the monomer.

Homopolymerization was run in anhydrous benzene at 80°C using 2,2'-azobisbutyronitrile as a free radical initiator. The polymer was isolated by precipitating the monomer from a benzene/methanol mixture and recovering the polymer by evaporating this solvent mixture. The IR-spectrum of this crude form showed the expected characteristic features of the polymer. The vinyl double bonds of the monomer disappeared and the melting temperature range broadened.

ACKNOWLEDGEMENTS

To appreciate my gratitude one has to know that I have ventured to attempt for a master's degree after closing my educational career 21 years ago. I owe my utmost gratitude to two personalities. But for the inspiration of my wife, Dr. Mujeebunisa (Taj) and the encouragement of my studies/thesis advisor Dr. Charles G. Gebelein, this dream would not have been a reality. During this period she kept my morale high and Dr. Gebelein rescued me at the troubled spots. I would also like to thank Dr. James A. Reeder and Dr. Thomas N. Dobbelstein for the valuable time they have spent reading and advising me in improving this report.

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CHAPTER I

GENERAL INTRODUCTION AND HISTORICAL

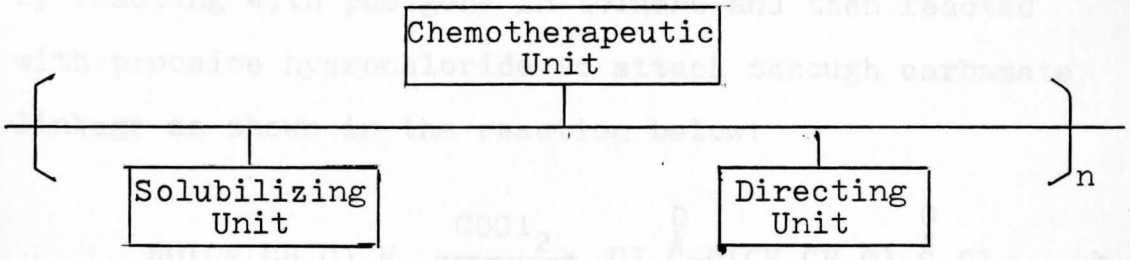
The use of polymers in the field of medicine is well known. Generally their applications are as replacement parts in the human body. The recent trend is in their application in the pharmacological field. Much interest has been shown lately in synthetic polymers for their biological activity as a means to increase the duration of activity of known drugs or as their carriers. The main aim is to achieve a low degree of toxicity, a high degree of specificity and prolonged duration of action.

The general line of approach is to modify a drug with a polymerizable group (eg. vinyl). The monomer thus obtained is polymerized (homo or co-) to a desired degree, so as not to lose its compatibility with the human physiology. An alternative route to achieve the same result is to attach the drug onto a known polymer. The drug can be attached to the polymeric back-bone or kept away from it by interposing some linear carbon chain molecules in between.

There are many ways to improve the characteristics of such a polymer. Its solubility in the blood might be increased by introducing some polar moieties to improve aqueous solubility or long chain hydrocarbons could be introduced to enhance the lipid solubility. There is also the possibility to include certain directing units that have affinity towards a specific organ or region of the body. Such a unit may guide the therapeutic polymer towards a specific target in the body and thus affect at this region exclusively. If successful, such systems can create a new generation of "miracle drugs".[2]

A chemotherapeutic polymer with some of its desirable functional groups can be schematically represented as in Figure #1, below:

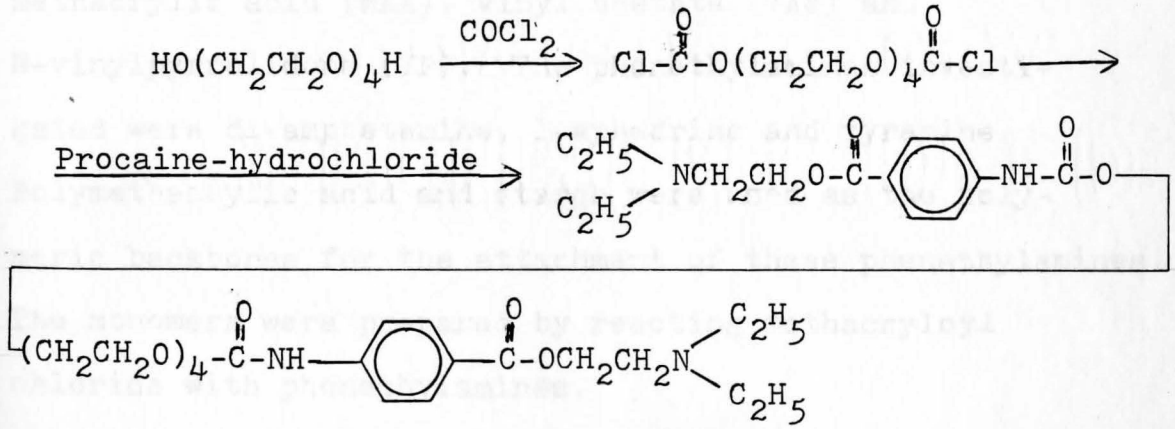
FIGURE #1



A known drug on a polymer chain may or may not retain its activity. One reason for loss of activity may be due to the proximity of the active unit of the drug to the polymeric chain. This can be remedied by interposing some linear carbon chain molecules and thus shift the active center away from the polymer chain.

It is also possible that the drug remains potent while it is attached to the polymer. Then it would have effectively a high concentration in a localized region and could serve as a long lasting drug until the polymer is degraded and/or removed from the system. On the other hand if the drug becomes potent only after its detachment from the polymer, it would still provide an opportunity to administer large doses of drug to last for a longer duration through controlled release of the potent drug in low concentrations.

Many researchers have worked along these lines. Weiner & Zilkha[3] attached the well known local anesthetic, procaine hydrochloride, to a known non-toxic polymer, polyethylene glycol and studied the possibility of its prolonged activity. The two terminal hydroxyl groups of polyethylene glycol were converted to chloro-carbonates by reacting with phosgene in toluene and then reacted with procaine hydrochloride to attach through carbamate linkage as shown in the reaction below:



Procaine derivative of polyethylene glycol

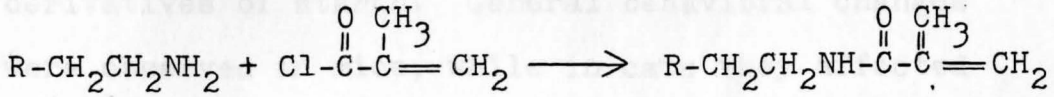
The procaine derivatives obtained were oils and soluble in ether, benzene and chloroform but insoluble in water. The procaine derivatives of tetraethylene glycol (TEG) and polyethylene glycol (PEG-400, MW-400) were tested for the anesthetic characteristics. The data is presented in Table 1 below:

TABLE #1

ANESTHETIC ACTIVITY OF PROCAINES[4]

Drug	Duration of Activity
1. Procaine hydrochloride.	15 minutes
2. PEG-400 Derivative.	45 minutes
3. TEG Derivative.	75 minutes

In another study Weiner, Tahan & Zilkha[5] modified phenethylamines with methacryloyl chloride to get N-meth-acryloyl derivatives of phenethylamines. These were then homo and co-polymerized with methacrylic acid (MAA), vinyl acetate (VAc) and N-vinylpyrrolidone (VP). The phenethylamines investigated were dl-amphetamine, l-ephedrine and tyramine. Polymethacrylic acid and starch were used as the polymeric backbones for the attachment of these phenethylamines. The monomers were prepared by reacting methacryloyl chloride with phenethylamines.



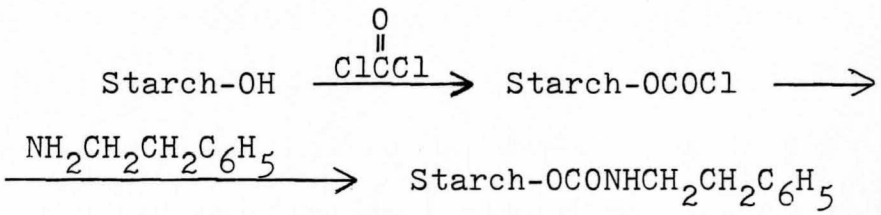
The monomers were polymerized in bulk, and the results are reported in Table #2.

TABLE #2

POLYMERIZATION OF N-METHACRYLOYL- PHENETHYLAMINE

Monomer	Yield %	POLYMER Solvent for Recrystallization	Melting Range °C
Derivatives of			
1. Phenethylamine	92	$CHCl_3$-Et_2O	127-150
2. dl-Amphetamine	80	$CHCl_3$-Et_2O	142-156
3. l-Ephedrine	10	$CHCl_3$-Et_2O	143-162
4. Tyramine	98	Insoluble	245-300

Phenethylamines were also modified with starch. The products were produced by reacting phenethylamines with chloro-formate starch derivative:

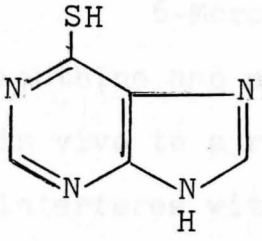


Preliminary pharmacological tests were carried out on monomeric N-methacryloyl phenethylamines, their polymers and co-polymers as well as on the phenethylamine

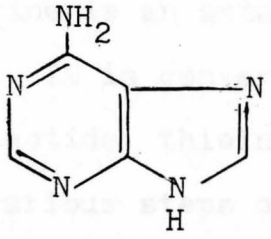
derivatives of starch. General behavioral changes were observed in mice, while in cats they affected the blood pressure. The most remarkable improvement is in the lethal dose (LD_{50}) level. For example the LD_{50} of homo or co-polymeric derivative of emphetamine increased to more than 1000 mg/kg compared against 25 mg/kg of the parent drug in mice. Further the mode of action of the polymeric compounds was sometimes in contrast to that of the parent drug. Thus the copolymer of N-methylacryloyl-amphetamine with vinyl-acetate showed depressant activity in contrast to amphetamine (stimulant), although it also increased blood pressure. In general the derivatives showed more desirable characteristics than the parent drugs.

MERCAPTOPURINE AS A DRUG[6]

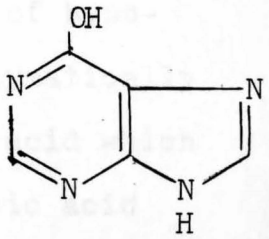
6-Mercaptopurine(I) was first synthesized and developed by Hitchings[7] and colleagues at Wellcome Research Laboratory in 1951. It is an analogue of nucleic acid constituents like adenine(II) and hypoxanthine(III) which are the physiological base forms of purine:



(I)



(II)



(III)

Being a very close analogue it was expected to be accepted by the tissue and thus interfere with the biosynthesis of nucleic acid. It was further hoped that it may be more damaging to the parasitic than to normal tissues, but tests showed no specific preferences. It is well absorbed and distributed evenly in the body but passes the blood brain barrier poorly. It undergoes catabolic destruction in vivo and is oxidized to methylated derivatives and is not detectable in the urine twelve hours after oral administration.

Early clinical investigations highlighted its usefulness in the treatment of acute leukemia and myelocytic leukemia. Temporary remission, either partial or complete, was achieved. In general it appeared that a higher proportion of children than adults responded favorably to the drug. It showed no effectiveness towards treatment of chronic leukemia, solid tumors and Hodgkins disease.

6-Mercaptopurine is an antagonist of hypoxanthine and adenine. It is converted enzymatically in vivo to a ribonucleotide, thioinosinic acid which interferes with the various steps of nucleic acid biosynthesis. Hitchings and Elion[7] studied its interfering mechanism. 6-Mercaptopurine and 6-methylthiopurine are converted to the respective ribonucleotides. These and the thioinosinic acid interfere with the conversion of inosinic acid to adenylic and guanylic acids and act as back inhibitor of purine biosynthesis steps. 6-Mercaptopurine is also incorporated into deoxyribonucloic acid in the form of thioguanine which interferes with the conversion of thioinosinic acid to thioguanylic acid. To date there is no known antagonist of 5-mercaptopurine. The physician has to be extra alert to discontinue the drug at the first symptom of toxicity as the drug

is known to have delayed action. Depression in bone marrow, large fall in white cell count and jaundice due to liver damage or biliary stasis are common toxic manifestations. Laboratory animals kept on high dosage suffered from bleeding, diarrhea, microscopic lesions, loss in weight, leukopenia and degenerative changes in the intestinal epithelium and liver.

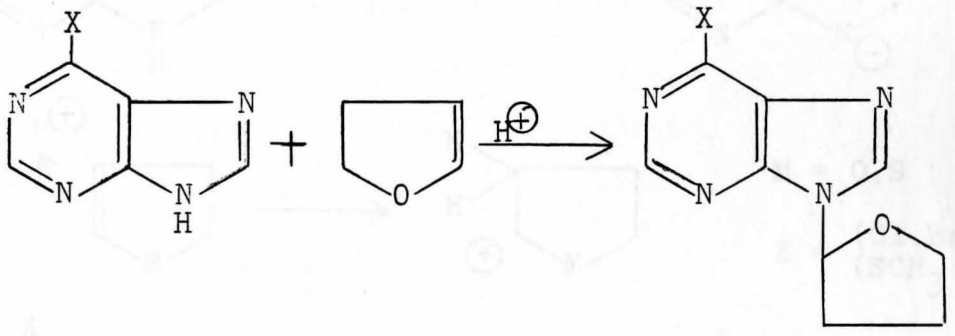
6-Mercaptopurine showed good remission in patients who become resistant to cortisone. Similarly those who became resistant to 6-mercaptopurine subsequently responded to cortisone. Hence in the treatment of acute leukemia, 6-mercaptopurine is the drug of choice with the steroids being reserved for emergencies where resistance to antimetabolites has developed. By use of these drugs in proper sequence definite increase in survival time can be achieved.

EFFECT OF SUBSTITUTION ON THE ACTIVITY OF 6-MERCAPTOPURINE

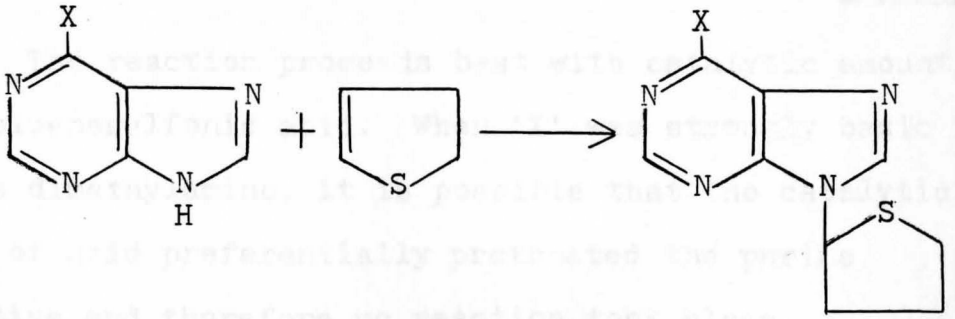
Among the purines, 6-mercaptopurine and 6-chloro-substituted derivatives show anticarcinogenic characteristics. The 6-substituted purines have only one reactive center (7 or 9) in the purine ring. Among the two tautomers the 7-H exists in traces, but it is possible to obtain 7-substituted compounds.[8] 9-Alkyl-substituted 6-mercaptopurine and 6-chloropurine retain considerable amount of antitumor activity possessed by their parent 6-substituted compounds, but the 7-alkyl-substituted corresponding purines are devoid of the antitumor activity.[9] It seemed possible that the 9-substituted purines might owe their activity to the natural relationship to purine nuclosides rather than to possible demethylation in vivo. This is supported by yet another comparative study where a planar phenyl group[10] in the 9-position shows no activity while a tetrahdropyran ring system (which resembles natural purine nucloside more closely) exhibits significant antitumor activity.

FURANOSIDE STRUCTURES WITH SUBSTITUTED PURINES

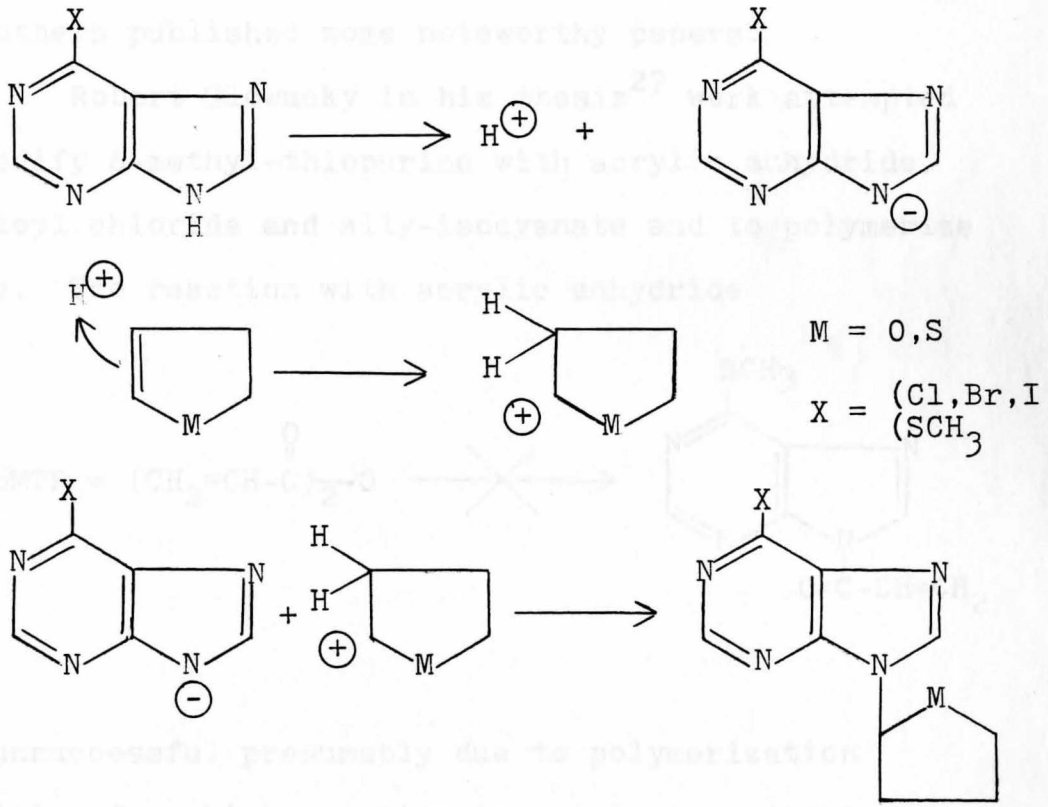
Lewis, Schneider and Robins[9] attempted to synthesize substituted purine nuclosides similar to naturally occurring purine nuclosides. They found that 2,3-dihydrofuran, in the presence of catalytic amounts of acid, readily reacts with most 6-substituted purines like 6-(halo)purine, 6-cyanopurine, 6-methylthiopurine or other 6-substituted purine to form corresponding 9-(tetrahydro-2-furyl)-6-substituted purine derivatives.



However, the substitution with 2,3-dihydrothiophene in position-9 is not as readily carried out under these reaction conditions.



A study of the general reaction of 2,3-dihydrofuran and 2,3-dihydrothiophene with various 6-substituted purines revealed that the reaction proceeded best if the substituent in position-6 was an electron withdrawing group. The reaction failed when the substituent at position-6 was -H, $-NH_2$, -OH, -SH or dimethylamino groups. These experimental observations suggest the reaction mechanism to be

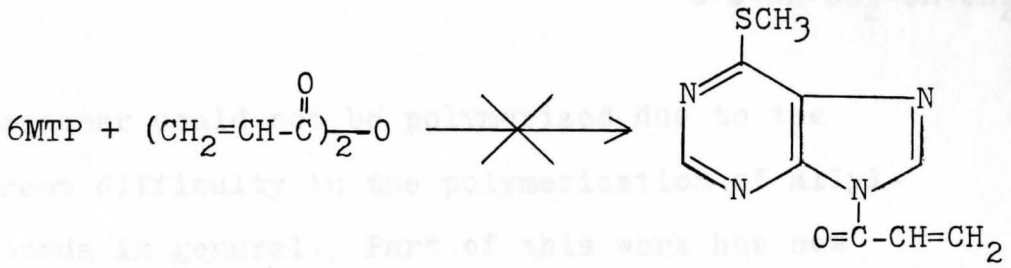


The reaction proceeds best with catalytic amount of p-toluenesulfonic acid. When 'X' was strongly basic such as dimethylamino, it is possible that the catalytic amount of acid preferentially protonated the purine derivative and therefore no reaction took place.

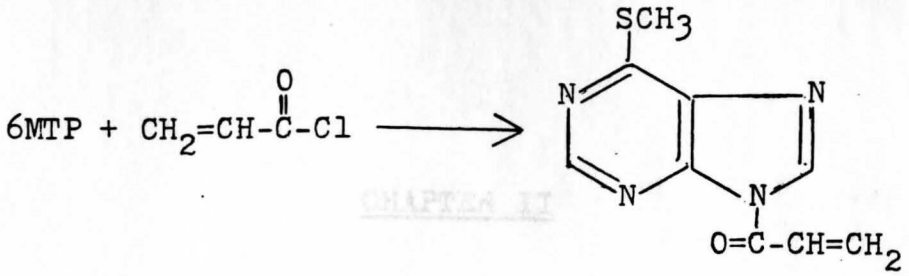
PREVIOUS WORK

Keeping abreast with the recent trend Dr. Charles G. Gebelein developed interest in the field of chemotherapeutic polymers[25] and concentrated his efforts towards two well known anticarcinogenic drugs, 6-mercaptopurine and 5-fluorouracil. He along with his graduate students Robert Glowacky,[27] A. Baytos,[28] Richard M. Morgan,[29] Timothy Ryan,[30] and Mirza W. Baig[31] and others published some noteworthy papers.

Robert Glowacky in his thesis[27] work attempted to modify 6-methyl-thiopurine with acrylic anhydride, acryloyl chloride and ally-isocyanate and to polymerize these. The reaction with acrylic anhydride

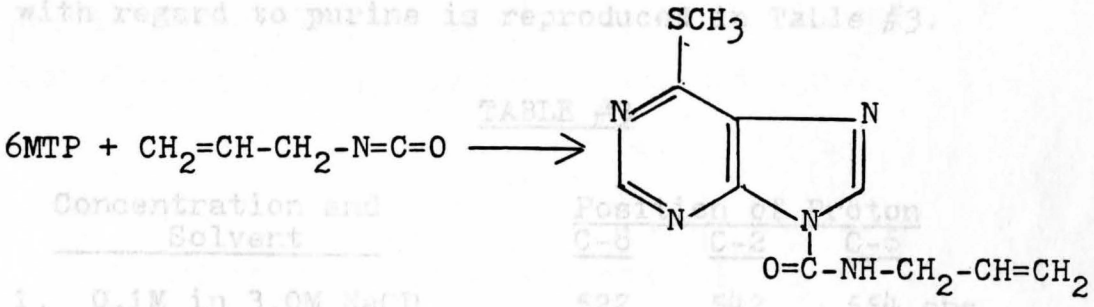


was unsuccessful presumably due to polymerization resulting from high reaction temperatures. Acryloyl chloride did show



evidence of reaction but the product could not be purified adequately.

The most successful reaction was between 6-methylthiopurine and allyl-isocyanate.



The monomer could not be polymerized due to the inherent difficulty in the polymerization of allyl-compounds in general. Part of this work has now been published.[27] A. Baytos studied the addition of iodine isocyanate to polyisoprene and the subsequent reaction with sulfanilamide to form a potentially-active polymer.[28] R. Morgan and T. Ryan studied the reaction of various unsaturated isocyanates with 5-fluorouracil to form new monomers containing this chemotherapeutic group.[29,30] Part of this present work has also been published.[31]

CHAPTER II

SURVEY OF ANALYTICAL STUDIES ON PURINES

A. NUCLEAR MAGNETIC RESONANCE

Bullock and Jardetzky[11] made NMR studies of the chemical shifts of C-2, C-6 and C-8 protons of purines and 6-substituted purines in NaOD, D_2O and D_2SO_4 solutions on a 60MHz instrument. Their findings with regard to purine is reproduced in Table #3.

TABLE #3

Concentration and Solvent	Position of Proton C-8	C-2	C-6
1. 0.1M in 3.0M NaOD	522	542	554 cps
2. 0.1M in D_2O	527	545	555 cps
3. 0.1M in 0.6M D_2SO_4	563	579	589

They could correctly assign the position by comparing the spectra of purine, 6-deuteriopurine and 8-deuteriopurine. The position of C-2, C-6 and C-8 protons of 0.1M respective purines in D_2O is presented in Table #4.

TABLE #4

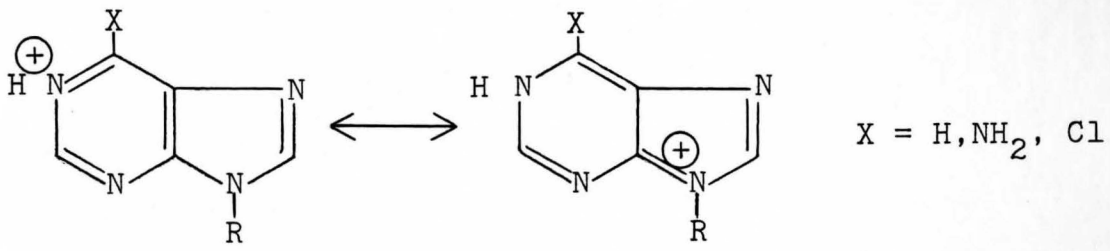
	Position of Proton (cps)		
	C-8	C-2	C-6
1. Undeuterated Purine	527	545	555
2. 6-Deuteriopurine	527	545	Absent
3. 8-Deuteriopurine	Absent	454	555

Irrespective of the acidity of the solution the C-8 proton peak of purine always appears at a higher field than C-2. In 6-substituted purines, however, it is dependent on the pH of the solution. The C-8 proton appears at higher field than C-2 proton in basic solutions and crosses over to lower field in acidic solutions.

The solvent effect on chemical shift[12] of the protons in purines and 6-substituted purines has been reported by Hruska, Bell et al.[12] The chemical shift of C-2, C-6 (methyl) protons are essentially solvent independent but there is a large variation in the C-8 proton in non-aqueous media. The C-8 proton shifts to low field in proton acceptor solvents and the shift is proportional to the relative proton acceptor strength of the solvent. The chemical shift of the C-8 proton has been attributed to its capability to form hydrogen-bonds both in aqueous and non-aqueous media,

whereas the C-2 and C-6 proton show no such tendency. This is supported by molecular orbital calculations,[13] and dipole measurements[14] which indicate that C-8 carbon is the most electron deficient center in purine and hence possesses some acidic character giving tendency toward hydrogen-bonding.

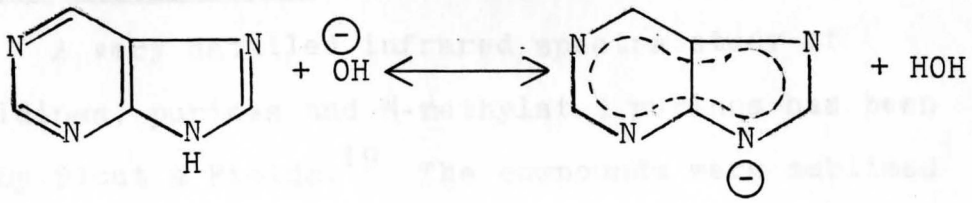
Several studies [15,16,17] indicate that many purines are protonated in the pyrimidine ring, most probably at N-1. Also the greater chemical shift of the C-8 proton compared to C-2 and C-6 protons in acid solutions suggest that there is some delocalization of the charge into the imidazole ring (resulting in cross-over as mentioned above). Hence it is very likely that the cation exist as a linear combination of resonance structures I and II.



(I) (II)

X = H, NH_2, Cl

Appearance of C-8 proton at higher field than C-2 and C-6 protons can be explained in terms of purine anion formation. Since the N-9 (N-7) proton has acidic character,[18] dissolution in basic solution would tend to favor the anionic form which can be represented by the equilibrium



In the anionic form the excess electron density is presumably redistributed via the π-electron system throughout the entire molecule and thereby leads to an increased shielding at each position on the ring.

B. INFRA-RED ANALYSIS

A very detailed infrared spectra study of pyrimidines, purines and N-methylated purines has been made by Blout & Fields.[19] The compounds were sublimed on rock salt under high vacuum and scanned in the 670-5000 cm^{-1} region.

In pyrimidine class cytosine, uracil and thymine were investigated. The bands at 3425 & 3205 cm^{-1} were assigned to free OH and NH_2 stretching vibrations,[20] respectively. They suspected the band at 2817 cm^{-1} to be due to the C-H stretching, but later workers[21] assigned it to ring N-H. The displacement to lower frequencies than usual for $-NH_2$ and O-H bands was attributed to inter-molecular hydrogen bondings. Cytosine registers an intense peak at 1661 cm^{-1} (C=C & C=N stretching motion), uracil shows a doublet at 1709 and 1695 cm^{-1} (C=C & C=N or C=O stretch motion) and thymine gives two distinct bands at 1761 cm^{-1} (C=O stretching motion) and 1678 cm^{-1} (C=C and C=N stretching motion). The band at 1460 cm^{-1} in all three represent the C-H bending. The $C-CH_3$ vibration causes the 1381 cm^{-1} band in thymine. The bands 1282, 1227 and 1242 cm^{-1} in cytosine, uracil and thymine respectively are due to C-N vibrations. Similarly the bands 1242 and 122- cm^{-1} in cytosine and thymine correspond to C-O vibration.

Adenine, guanine, hypoxanthine and xanthine were investigated from the purine group. The IR-spectra of the four show some very close similarities. The C=C stretching mode appears in the 1701 to 1672 cm^{-1} range and the C=N stretching in the 1608 to 1558 cm^{-1} region. The bands in the region 2941 to 2703 cm^{-1} were again mistakenly assigned to C-H stretching which later workers[21] found to be due to N-H stretching. The NH_2 of adenine and guanine appears at 3300 cm^{-1} which is absent in hypoxanthine and xanthine. The band at 3125 cm^{-1} represents O-H stretching in guanine, hypoxanthine and xanthine.

Among the N-methylated purines theophylline, theobromine and caffeine were analyzed. The absorption in the 3300 cm^{-1} region shows no abnormalities compared to other purines. The band at 2618 cm^{-1} in theophylline is associated with C-H stretching motion. Theophylline and caffeine show the C=O stretching motion bands at 1706 cm^{-1}.

Bryant & Harmon's[1] investigations showed that the band at 3077 cm^{-1} in 6-alkylthiopurine is due to N-H absorption at the 9-position which disappears in the 9-H substituted 6-alkylthiopurine derivatives.

Novac & Lautie's[21] work is confined to the N-H (9-position) stretch bands in purines. There are ten relatively well defined and strong subbands in the range 2525 to 3075 cm^{-1} and 40-270 cm^{-1} (Far Infra-red) region associated to the N-H (9-position). This is confirmed by the IR disappearance of the bands from the spectrum when the 9-H is deuterated. Their frequencies are recorded in Table #5.

TABLE #5

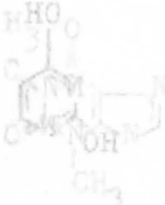
ABSORPTION BANDS OF N-H (9-POSITION) IN PURINE[21]

Infra-Red Frequencies cm^{-1}	Far IR Frequencies cm^{-1}
3070	268
3010	230
2941	168
2865	130
2780	110
2725	91
2680	73
2610	57
2557	51
2538	41

All the data[1,19,21] put together gives very clear spectra of the pyrimidines, purines and N-methylated purines which is collected in the Table #6.

TABLE #6

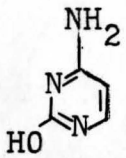
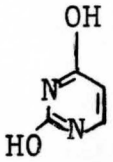
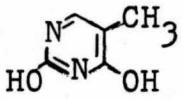
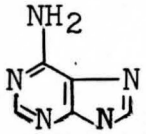
IR ABSORPTIONS OF SOME METHYLATED PURINES (cm^{-1})

GROUPS	Caffeine	Theo-phylline	Theo-bromine	Xanthine	Hypo-xanthine	Guanine	Adenine
							
1. C-CH$_3$	--	--	--	1381	--	--	--
2. C-O	1542	1552	1552	1550	1550	1550	1550
3. C-NH	1625	1627	1627	1627	1627	1627	1627
4. C=N	1453	1453	1453	1460	1460	1460	1460
5. C=O	--	--	1706	1761*	--	--	--
6. C=N or C=O	1697	1697	Double 1709 & 1695	Mixed 1678	Mixed 1678	Mixed 1678	Mixed 1678
7. C=N	1691	1691	1691	1701	1701	1701	1701
8. N-H	2817	2819	2819	2819	2819	2819	2819
9. NH$_2$	3205	--	--	--	--	3300	3300
10. O-H	2925	2925	2925	2925	2925	3125	3215

*In tautomeric form.

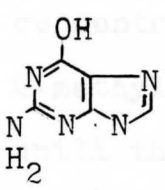
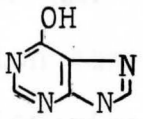
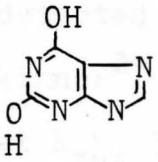
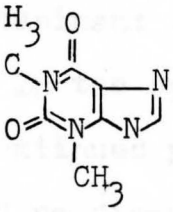
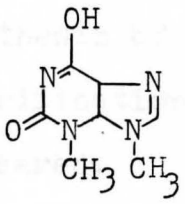
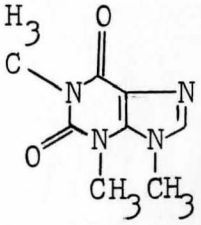
TABLE #6

IR. ASSIGNMENTS OF PYRIMIDINES,

	1	2	3	4
Name & Structure	Cytosine 		Thymine 	
GROUPS		Uracil		Adenine
1. C-CH_3	--	--	1381	--
2. C-O	1242	Mixed 1227	1220	--
3. C-N	1282		1242	1250
4. C-H	----------	----------	1460	----------
5. C=O	--	--	1761*	--
6. C=N or C=O	Mixed 1661	Doublet 1709 & 1695	Mixed 1678	1587 ---------
7. C=C				1672 --------
8. N-H	2817	2899	3175	--------
9. NH_2	3205	--	--	3300
10. O-H	3425	3205	3247	--

*In tautomeric form.

PURINES AND N-METHYLATED PURINES (CM^{-1})

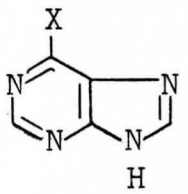
5	6	7	8	9	10
Guanine 	 Hypo-Xanthine	Xanthine 	 Theo-Phylline	Theo-Bromine 	 Caffeine
--	--	--	--	--	--
1176	1212	1212	--	1227	--
1258	1258	1316	1285	1295	1287
----------	----------	1427	1445	1458	1443
--	--	--	1706	--	1706
[1608	1587	1558]	1613	1597	1603
[-------1608/1558----------]					
[1672	1672	1701]	1672	1672	1661
[-------1701/1672----------]					
-------3075/2525----------			2618	--	--
3300	--	--	--	--	--
3215	3125	3125	--	3175	--

C. ULTRA-VIOLET SPECTROSCOPY.

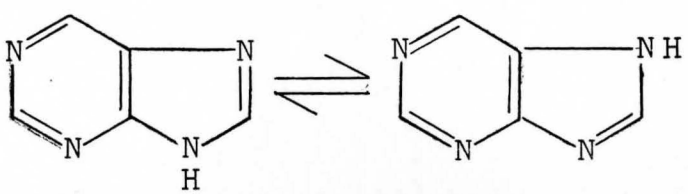
Ultra-violet spectroscopy is a very useful technique to establish purity of purines. 6-Mercaptopurine absorbs at 328 nm[24] while 6-alkylthiopurines absorb at λ_{max} 288-292 nm. Traces of contaminant in 10^{-4}M concentration can be detected. In the synthesis of 6-methylthiopurine, Skinner[24] continued purification until the absorption at λ_{max} 328 nm disappeared. Table #7 provide some data on UV absorption of 6-substituted purines.

TABLE #7

UV. ABSORPTION OF 6-SUBSTITUTED PURINES

	λ_{max} nm	ϵ	Solvent	Ref:
X= SH	328	x	95% EtOH	24
= S(Alkyl)	288/292	17900/22000	95% EtOH	1
= NH_2	268	12,000	.05N NaOH	19
= OH	263	10,700	0.1N NaOH	19

The purine structure is in a tautomeric state which is represented by:



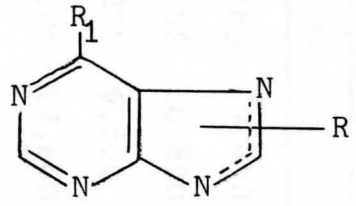
(I) (II)

The contribution of structure (II) is very minor with the result some minute quantities of 7-substituted isomers might occur in the synthesis of 9-substituted derivatives of purines. Greenburg[10] and Prasad[8] studied the UV. absorption of 7-methyl and 9-methyl derivatives of 6-substituted purines. In general 7-substituted isomers absorb at higher wavelength than 9-substituted compounds. Lewis[9] utilized the above data, as comparison, to assign the location of tetrahydro-2-furyl and tetrahydro-2-thienyl groups to position-9 in 6-substituted purines as presented in Table #8.

The effect of pH on UV. absorption was checked by Lewis. The data is reproduced in Table #9.

TABLE #8

ULTRA-VIOLET ABSORPTION OF SOME 9-(TETRAHYDRO-2-FURYL) AND 9-(TETRAHYDRO-2-THIENYL)-6-SUBSTITUTED PURINES AND RELATED 7- AND 9-METHYLPURINES

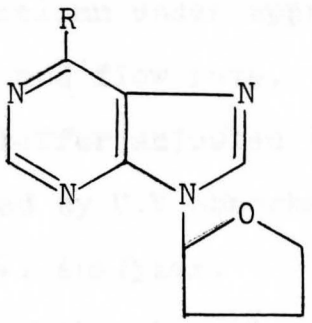


R_1	7-Methyl[8] nm		9-Methyl[10] nm		9-(Tetrahydro-2-furyl) nm		9-(Tetrahydro-2-Thienyl) nm	
Cl	271	7,300	265	9,100	266	9,000	265	9,300
NH_2	272	9,500	262	12,500	261	13,900	x	x
SCH_3	293	14,000	284	17,800	284	19,100	284	20,600
OH	257	9,150	249	10,200	x	x	249	11,100

TABLE #9

UV. ABSORPTION OF SOME 9-(TETRAHYDRO-2-FURYL)

-6-SUBSTITUTED PURINES



		pH 1		pH 11		Ethanol	
#	R	nm		nm		nm	
1.	Cl	265	9,400	266	9,600	266	9,000
2.	Br	266	10,800	267	11,300	267	11,600
3.	I	277	9,800	277	11,400	275	11,400
4.	SH	326	17,600	312	20,400	326	17,100
5.	SCH_3	295	14,400	290	17,700	284	19,100
6.	$o\text{-}FC_6H_4CH_2S$	294	17,500	293	21,800	285	20,800
7.		281	16,000	290	18,500	284	20,400
8.	NH_2	263	15,200	261	17,200	261	13,900
9.	$N(CH_3)_2$	277	14,400	276	18,700	275	15,800
10.	$NHCH_2CH_2OH$	273	15,700	268	17,200	268	10,700
11.	$HNCH_3$	267	15,100	267	16,400	266	16,000
12.	CN	289	9,000	289	9,500	288	9,100

D. CHROMATOGRAPHY

Purines and pyrimidines can be isolated efficiently by chromatographic technique. Excellent separation has been achieved by Sweetman & Nyhan[22] with a Sephadex G-10 column under appropriate conditions of pH, ionic strength and flow rate. The eluent used was 0.5M $NaHPO_4$ buffer adjusted to pH 7.0 with NaOH. It was monitored by U.V absorbance with a Vanguard Automatic U.V. Analyser.

The recovery of the chromatographed purines was quantitative (99% or more) with excellent reproducibility. Regeneration was not necessary after use of the column for most runs. Some columns were repeatedly used for a year without regeneration. If necessary, regeneration can be done with several column volumes of 0.05N NaOH solution followed by buffer of pH 7.0.

About 90 purines, pyrimidines and related compounds from biological fluids were isolated and studied. An extensive work has been reported with regard to the elution volumes, effect of flow rate, ionic strength and the calculated height equivalent to a theoretical plate (HETP). The data provides a relationship between elution volume, compound structure and the effect of substituents.

The particle size of Sephadex G-10 was 40-120 μ and mass to volume ratio was high. A slurry in aqueous buffer solution was poured into a 100 x 1 cm, LKB column and allowed to settle under buffer flow (3-4 psi) for several days. The void (outside the gel beads) volume (V_o) is determined by dyed blue dextran (Mol. wt. 2×10^6). An acetone/water solution gives the void volume plus the internal volume (V_i) that is accessible to small molecules. The elution volume (V_e) is determined from the time of application of the sample to the center of the peak on the chromatogram. A typical chromatogram is presented in Figure #2 below:

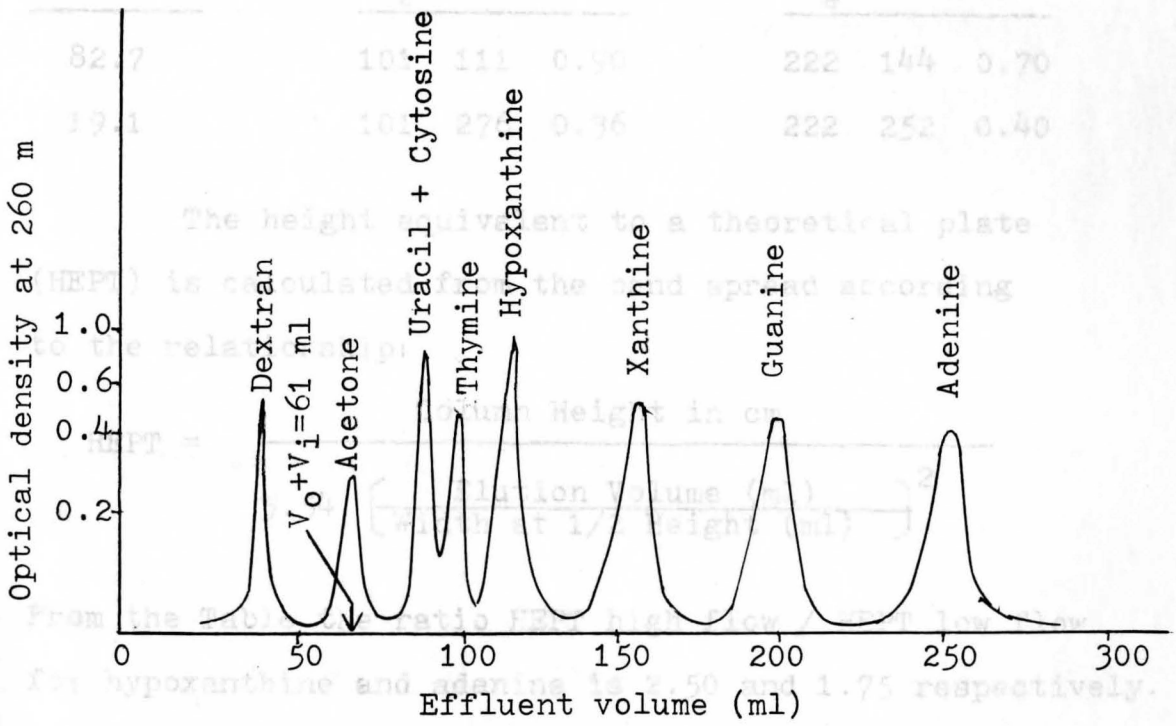


Figure #2. Chromatogram of purines and pyrimidines on 100 x 1.0 cm Sephadex G-10.

The elution volume is largely independent of sample volume and pH. At higher concentration tailing of the peak is observed while the elution volume remains unchanged. The effect of flow rate can be observed from Table #10.

TABLE #10

EFFECT OF FLOW RATE ON THE EFFICIENCY OF 1.0 x 100 cm G-10 SEPHADEX COLUMN

Flow Rate $ml/cm^2/hr$	Hypoxanthine			Adenine		
	V_e	H	HEPT	V_e	H	HEPT
82.7	101	111	0.90	222	144	0.70
19.1	101	276	0.36	222	252	0.40

The height equivalent to a theoretical plate (HEPT) is calculated from the band spread according to the relationship:

$$HEPT = \frac{\text{Column Height in cm}}{5.54 \left[\frac{\text{Elution Volume (ml)}}{\text{Width at 1/2 Height (ml)}}\right]^2}$$

From the Table the ratio HEPT high flow / HEPT low flow for hypoxanthine and adenine is 2.50 and 1.75 respectively. Therefore, the column is approximately twice as efficient at low flow rate and the peaks are sharper.

The partition coefficient, K_d, in Sephadex gel chromatography is defined as $K_d = (V_e - V_o)/V_i$ and is independent of column dimensions, but V_i is dependent on the size and structure of the molecule used to determine the internal volume (V_i) of the column. For corrolation purpose the elution volume (V_e) is corrected. The corrected elution volume (V_e^o) is given by the relation:

$$V_e^o = (V_e - V_o)/V_o = K_d(V_i/V_o)$$

that is

$$\log (V_e^o) = \log K_d + \log (V_i/V_o) = \log K_d + \text{Constant}$$

Some elution volumes (V_e) and corrected elution volume (V_e^o) are recorded in Table #11 for further consideration.

TABLE #11

ELUTION VOLUME OF SOME PURINES

#	Compound	V_e(ml)	V_e^o	log V_e^o
1.	Purine	106	2.12	+0.326
2.	6-Mercaptopurine	275	7.09	+0.851
3.	2-Aminopurine	187	4.77	+0.679
4.	6-Thioguanine	555	16.07	+1.206

A relation between elution volumes and purine structure emerges, when the above data is further analyzed as in Table #12.

TABLE #12

ELUTION VOLUME vs. PURINE STRUCTURE

Difference of log V_e^o of Compounds in Table #9	$=\Delta \log V_e^o$	Attributable to Substituent
2 & 1	+0.525	6-SH
4 & 3	+0.527	6-SH
3 & 1	+0.353	2-NH_2
4 & 2	+0.355	2-NH_2

It may be assumed from the above data that each substituent in a molecule contributes a fixed fraction of the difference in $\Delta \log V_e^o$ value, independent of the other groups present in the molecule. The assumption is analogous to the treatment of the ΔR_M function applied by Bush[23] to the R_F values of steroids in paper chromatography. The usefulness of the assumption is indicated by an example, 6-thioguanine (6-mercapto-2-amino-purine), whose elution volume can be calculated as follows:

$$\log V_e^o \text{ (6-thioguanine)} = \log V_e^o \text{ (purine)} + \Delta \log V_e^o \text{ (6-SH)} + \Delta \log V_e^o \text{ (2-NH}_2\text{)}$$

$V_e = (V_e^o + 1)V_o$, where V_o = 32.5 ml. from Tables # 11 & 12

$\log V_e^o$ (purine)	= +0.326
$\Delta \log V_e^o$ (6-SH)	= +0.525
$\Delta \log V_e^o$ (2-NH_2)	= +0.353
$\log V_e^o$ (6-thioguanine)	= +1.204

Therefore, V_e^o (6-thioguanine) = 16.0 and V_e = 552 ml. This predicted value may be compared to the experimental V_e = 555 ml in Table # 11. Thus the determination of $\Delta \log V_e^o$ values of various chemical groups located at different positions on the purine ring makes it possible to predict elution volumes of compounds containing many groups. The $\log V_e^o$ values of purines and their ribosides reflect the contribution of a ribosyl group in the 9-position of the purine ring to the adsorption to Sephadex-10, and give confirmation of the validity of the assumptions.

Sephadex-10 is an excellent adsorbent for the separation of many purines, particularly the methyl isomers. It promises to be very useful for preparatory work in purine synthesis where products can be separated from starting materials. Adequate separations from biological fluids are obtained at flow rate of 24.4 $ml/cm^2/hr$ in 24 hours with a 100-cm column.

CHAPTER III

STATEMENT OF THE PROBLEM

This work is in line with the recent trend towards incorporating pharmacologically active compounds onto a polymeric back-bone. In general such modifications impart specificity, lower toxicity and prolonged duration of activity. The drug of interest is 6-mercaptopurine, which was once widely used as antineoplastic drug for the treatment of acute leukemia. More recently it is used less due to its high toxicity and non-specificity towards abnormal cells.

In the earlier work Glowacky[31] tried in vain to incorporate this drug, 6-mercaptopurine onto a poly-isoprene polymer. He then attempted to modify acrylic and allylic monomers with 6-mercaptopurine with an intention to polymerize in the following stage. But unfortunately neither of his attempts were completely successful.

The present assignment is to react vinyl-isocyanate with 6-mercaptopurine, purify this monomer [6-methylthio-9-(N-vinylcarbamoyl)purine] and polymerize it.

CHAPTER IV

EXPERIMENTAL

Reagents.

The following chemicals were used in this research work. All these chemicals were used without any further purification. The physical constants of some chemicals used in this study are shown in Table #13.

1. 6-Mercaptopurine monohydrate (Aldrich)
2. Acryloyl Chloride (Polyscience Inc.)
3. Methyl Iodide (J. T. Baker)
4. Sodium Azide (Matheson Coleman & Bell) (Fisher Scientific)
5. Hydroquinone (Stansi Scientific)
6. Triethylamine (Eastman)
7. 2,2'-Azobisbutyronitrile (Aldrich)

Anhydrous Benzene

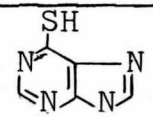
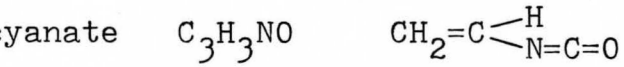
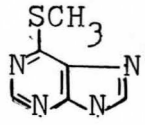
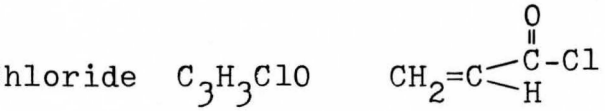
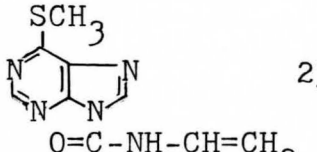
Benzene (1.0 l) was first dehydrated with $CaCl_2$ (50.0 g) for 24 hours. It was decanted, refluxed for 24 hours with sodium metal (10.0 g) and distilled.

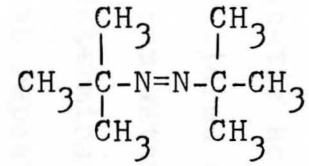
Purified AIBN

Excess of AIBN was dissolved in methyl alcohol at room temperature and filtered. On cooling in a refrigerator for 24 hours, needle like crystals separated out. It was decanted and rinsed with cold methanol and air dried.

TABLE #13

PHYSICAL CONSTANTS

#	Compound	Emperical Formula	Structure	Molecular Weight	Range °C Melting	Range °C Boiling
1.	6-Mercaptopurine	$C_5H_4N_4S$		152.0	313/314	-
2.	Vinyl-isocyanate	C_3H_3NO		69.0	-	39
3.	6-Methylthio-Purine	$C_6H_6N_4S$		166.0	218/220	-
4.	Acryloyl Chloride	C_3H_3ClO		90.5	-	73/76
5.	6-Methylthio-9-(N-Vinylcarbamoyl) Purine	$C_9H_9N_5OS$		235.0	169,d	-
6.	Methyl Iodide	CH_3I	CH_3I	142.0	-	42.5

7.	AIBN	$(C_4H_6N_2)_2$		164.0	103,d	-
8.	Sodium Azide	NaN_3	Na-N=N=N	65.0	-	-
9.	Triethylamine	$C_6H_{15}N$	$(C_2H_5)_3N$	101.0	-	89.3

Procedure for the Preparation of 6-Methylthiopurine

6-Mercaptopurine was first converted to 6-methyl-thiopurine to ensure the modification at the 9-position by the method adopted by Elion, Burgi and Hitchings.[7] 6-Mercaptopurine 11.55 g (12.95 g for monohydrate), (0.076 mole), was completely dissolved in 40.0 ml of 2.0 N-NaOH solution in a 250-ml three-necked flask. It was diluted with 75.0 ml water and stirred with an electric stirrer. While stirring, 11.0 g (.078 mole) methyl iodide was slowly added. After two hours of stirring at room temperature the mixture was cooled, the pH was adjusted to 5.0 with acetic acid and the resultant colorless needles were filtered off. This was purified by recrystallization from water and dried over night at 120°C for the determination of melting point. Recrystallization was repeated until the melting point values matched with the reported[7] 218-220°C range. Five batches of 6-methylthiopurine were made which are reported in Table #14. Its IR. spectra is marked as Figure #4.

TABLE #14

THE PREPARATION OF 6-METHYLTHIOPURINE USING 0.076M 6-MERCAPTOPURINE, 0.078M METHYLIODIDE IN A 0.7N NaOH SOLUTION

Batch #	Yield 6-Methylthiopurine	
	Grams	%
1	5.93	47.00
2	7.31	57.94
3	8.57	67.93
4	8.63	68.41
5	8.66	68.64
literature[7]	8.90	70.55

The Procedure for the Preparation of Vinyl-isocyanate

Vinyl-isocyanate was prepared by the Butler & Monroe[32] procedure. Into a 500-ml, three-necked flask, equipped with a reflux condenser fitted with a calcium chloride drying tube, a mechanical stirrer, thermometer, and an additional funnel, were placed 35.0 g (0.538 mole) of purified sodium-azide(NaN_3), 100 ml water and 0.3 g of hydroquinone. The reaction vessel was in an ice-water bath, and a mixture of 33.0 g (0.365 mole) of acryloyl chloride and 100 ml of benzene was added at such a rate that the reaction temperature remained

at 10-15°C. The reaction was cooled to 0°C and stirred for six hours. The organic layer was removed and dried over calcium chloride for 24 hours. The dried azide solution and 0.3 g of hydroquinone were added to 300 ml of dry benzene in a 500-ml three-necked flask equipped with a magnetic stirrer bar, a thermometer and dry-ice/acetone Dewar condenser guarded by a calcium chloride drying tube. The reaction mixture was heated while stirring to 70/80°C until the evolution of nitrogen had ceased. The crude product was distilled through a 12" Vigreux column fitted with an ice-water condenser backed by dry-ice/acetone trap. The latter was guarded by a calcium chloride drying tube. The distillation was continued until the vapor temperature of 80°C was reached. The distillates in the receiving flask and dry-ice/acetone trap were combined and 0.3 g hydroquinone added and then redistilled, until the vapor temperature reached 39°C, through a 12", helix-packed column into a 50-ml flask immersed in a dry-ice/acetone bath.

Acryloyl chloride and vinyl-isocyanate are severe lachrymators. Strict precautions were taken to handle them in an efficient fume hood. Containers used were rinsed with aqueous ammonia before taking them out of the fume hood.

Three batches of vinyl-isocyanate were made which has been reported in Table #15.

TABLE #15

THE PREPARATION OF VINYL-ISOCYANATE FROM 0.538M SODIUM AZIDE AND 0.365M ACRYLOYL CHLORIDE

#	Vinyl-Isocyanate g	Yield %
1	11.95	47.5
2	18.32	72.8
3	15.16	60.2
literature[32] (a)	40.0	53.0

(a) 1.631m sodium azide and 1.105m acryloyl chloride

The Procedure for the Preparation of 6-Methylthio-9-(N-Vinylcarbamoyl) Purine

The monomer 6-methylthio-9-(N-vinylcarbamoyl) purine was synthesized by the general procedure developed by Dyer & Bender[33] for the preparation of carbamoyl derivatives of 6-methylthiopurine.

A mixture of 1660 ml dry benzene, 1.0 mole (166.0 g 6-methylthiopurine, 2.0 moles (139.0 g) vinyl-isocyanate and 2% triethylamine, on the weight of 6-MTP, was stirred at room temperature in a 2.0-l Erlenmeyer flask. To protect it from moisture, the neck of the Erlenmeyer flask was sealed and stirring was done magnetically. The reaction was carried for more than 12 hours to ensure complete reaction. The product, insoluble in benzene, was separated by filtration. The purification is discussed in the next section. The three batches made have been tabulated in Table #16.

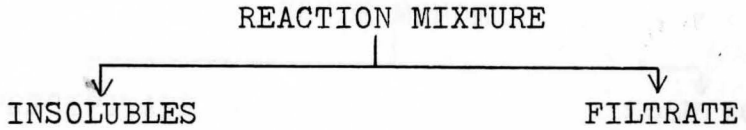
TABLE #16

PREPARATION OF THE MONOMER

Particulars	RUN # 1 Mole	1 Qty.	2 Mole	2 Qty.	3 Mole	3 Qty.
6-Methylthiopurine (g)	0.10	16.6	0.11	18.0	0.1	16.6
Vinyl-Isocyanate (g)	0.17	11.7	0.22	15.0	0.2	13.8
Triethylamine (g)	--	0.33	--	3.6	--	0.33
Benzene (Anhydrous) (ml)	--	1660	--	1800	---	1660
Reaction Time (hrs)	156		120		144	
Crude monomer (g)	18.5		30.09		20.6	
Theoretical yield (g)	23.5		25.85		23.5	
Yield %	78.7		85.9		87.7	

The Recovery and Purification of the Monomer by Recrystallization

After the completion of the reaction the insolubles, mainly the unreacted 6-methylthiopurine, polymerized vinyl-isocyanate and other complexes in the reaction mixture were removed by filtration. The clear yellow filterate contained the monomer 6-methylthio-9-(N-vinylcarbamoyl)purine , some unreacted 6-methylthiopurine, excess vinyl-isocyanate and triethylamine. The solvent, excess vinyl-isocyanate and triethylamine were removed by evaporation under vacuum.



Treatment of the Insolubles.

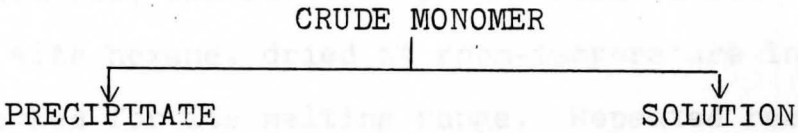
The insolubles were dispersed in water and filtered. White needle like crystals separated out from the filtrate on long standing. On further purification the crystals matched the melting point of 6-methylthiopurine (218-220°C).

Treatment of the Filtrate

The clear yellowish filterate was evaporated under vacuum and dried over P_2O_5 for 24 hours under vacuum. The yellow powder was washed three times with anhydrous ether to remove triethylamine and vinyl-isocyanate

and dried over P_2O_5 under vacuum. Dyer & Bender[33] reported recrystallization of some isocyanate derivatives from a 50/50 mixture of chloroform/hexane. But our attempts on these lines resulted in frustration. However a mixture of methylene chloride/hexane showed some positive results.

The crude monomer was dissolved in a minimum amount of methylene chloride (CH_2Cl_2) and precipitated with hexane. A yellowish sticky precipitate separated out.



Treatment of the Precipitate

The yellow sticky precipitate was removed by decantation, dissolved in CH_2Cl_2 and chilled at -80°C in an dry-ice/acetone bath for two days. Yellow crystals were formed which redissolved on allowing the solution to attain room temperature, leaving a brown resinous product at the bottom. The clear solution was decanted and subjected to the chilling and warming cycle as above. In the third cycle nothing separated out. The crystals which formed at -80°C could not be filtered at that temperature, as the crystals were very fine and dissolved with the slightest disturbance.

The brown resinous product was insoluble in methylene chloride, carbon tetrachloride, acetone, benzene, toluene, and hexane, but soluble in chloroform and dioxane. It started melting at 90°C and decomposed at 120°C to form opaque brown specks. The IR spectrum is presented in Figure #7, which resembles the IR Spectra (Figure #6) of the synthesized polymer.

Treatment of Solution

The light yellow solution was chilled in a dry-ice/acetone bath for two days. A white precipitate separated out, was filtered in the cold (about -80°C), washed with hexane, dried at room-temperature in vacuum, and checked for its melting range. Repeated recrystallization raised the initial melting point and narrowed the range which is elaborated in Table #17.

No improvement was recorded after the second recrystallization. The solvent system was changed to a chloroform/hexane mixture. Marked improvement was noted in Sample #IV and V. The elemental analysis report is presented in Table #18, and IR-Spectra on Figure #5.

The IR peaks match with those in the literature to identify the product as 6-methylthio-9-(N-vinylcarbamoyl) purine. The spectra will be discussed in detail in Chapter IV.

Three trials were made to purify by the process of repeated recrystallization. The details are in Table #19.

TABLE #17

MELTING RANGE OF MONOMER

Recrystallized Sample	Solvent 50/50	Melting Range °C
Ist	CH_2Cl_2/ Hexane	Started melting at 140°C, completes at 154°C. Remains liquid till 160°C and starts decomposing.
IInd	CH_2Cl_2/ Hexane	Range 157-160°C and decomposes at 160°C
IIIrd	CH_2Cl_2 Hexane	Same as above.
IVth	$CHCl_3$/ Hexane	166.5/168°C. Colorless, clear liquid, changes to yellow at 170°C and becomes darker with temperature.
Vth	$CHCl_3$/ Hexane	169/170.5°C, clear colorless liquid. Starts decomposing at 172°C and turns dark brown solid at 180°C.

TABLE #18

ELEMENTAL ANALYSIS REPORT

		Sample IV		Sample V	
Elements	Theoretical %	Actual %	Difference	Actual %	Difference
Carbon	45.96	45.14	-0.82	44.24	-1.72
Hydrogen	3.83	3.71	-0.12	3.68	-0.15
Nitrogen	27.79	29.11	-0.68	29.31	-0.48
Sulfur	13.62	12.76	-0.86	12.75	-0.87
Oxygen	6.80	9.28	+2.47	10.02	+3.22
Total	100.0	100.0	-	100.0	-

TABLE #19

MONOMER PURIFICATION BY REPEATED RECRYSTALLIZATION

	Product	Batch # I	II	III
1.	Soluble product from the reaction mixture.	--	30.09	20.6
2.	After extraction with anhydrous ether.	--	26.83	18.49
3.	Ist Recrystallization from 50/50 CH_2Cl_2/Hexane mixture.	--	15.40	16.14
4.	IInd Recrystallization from 50/50 CH_2Cl_2/Hexane mixture.	--	11.60	14.71
5.	IIIrd Recrystallization from 50/50 CH_2Cl_2/Hexane mixture.	--	9.30	11.73
6.	IVth Recrystallization from 50/50 $CHCl_3$/Hexane mixture.	--	2.76	9.94
7.	Vth Recrystallization from 50/50 $CHCl_3$/Hexane mixture.	0.3	2.15	7.02
8.	Yield	--	8.3%	29.9%

In conclusion, it is to be pointed out that:

1. The recrystallization process is very slow and cumbersome.
2. The yield is low (approx. 30%).
3. The desired purity is unattainable through recrystallization.
4. A more efficient technique needs to be employed.

Further Studies on Purification

The purification of monomer by the method suggested by Dyer & Bender[33] was found to be effective only at very low temperatures (-80°C). The recrystallization technique turned out to be impracticable and inefficient. To develop a better solvent mixture system to be useful at ambient temperature, the solubility of the monomer in about 35 laboratory solvents was determined. The results have been reported in Table #20. In general the monomer is soluble in solvents having solubility parameters in the range 8.3 to 12.1 (hildebrands). About a dozen combinations of solvents in different proportions were tried. But none was found to work as a recrystallization solvent at room temperature.

The solubility parameter (δ) is an approximate measure to predict the interaction of one substance with another in the process of dissolution. The process of dissolution is similar in many ways to the process of vaporization. Hence the solubility parameter (δ) is a derivation from the molar heat of vaporization[37] (ΔH_v) and the density. It is given by the equation:

$$\delta \cong \frac{(\Delta H_v - RT)^{\frac{1}{2}}}{M/d}$$

R - Gas constant
ΔH_v - Solvent heat of vaporization
M - Molecular weight of solvent
d - Density of solvent.

The units are $(cal/ml)^{\frac{1}{2}}$ and termed as 'hildebrands'.

Two substances have the probability to form a solution if their solubility parameters are close to one another. The prediction is very vague since the dissolution is dependant upon many other factors such as the interaction arising from the polar, non-polar effects and hydrogen bonding.

In the absence of availability of molar heat of vaporization of a solvent, the solubility parameter can be determined by other physical constants of the solvent. Some of the relations are quoted here.

a) From thermal coefficients

$$\delta \cong \left[\frac{\alpha T}{\beta}\right]^{\frac{1}{2}}$$

α - Thermal expansion coefficient

β - Compressibility

b) From van der Waal's Gas constant

$$\delta \cong \frac{1.2\ a^{\frac{1}{2}}}{V}$$

a - van der Waal's constant

V - Molar volume

c) From Critical Pressure

$$\delta \cong 1.25\ P_c^{\frac{1}{2}}$$

P_c - Critical pressure

d) From Surface Tension

$$\delta = 4.1 \left[\frac{\gamma}{V^{1/3}}\right]^{0.43}$$

γ - Surface Tension

V - Molar volume

e) From Structural Formula

Solubility parameter has additive properties. Each atom or group 'G_i' in a molecule 'G' contributes its share to the total. It is given by the equation:

$$\delta = \frac{d \sum G_i}{M}$$

$\sum G_i$ - Molar attraction constant of the group

d - Density of solvent

M - Mol. Wt. of solvent

This method is very useful for determining the solubility parameter of polymers.

Since most polymers decompose before evaporation, the solubility parameter must be determined indirectly by comparison with that of a suitable solvent. The polymer is treated with a series of solvents of different solubility parameters. The extent of solubility or swelling is plotted against the solubility parameters of the solvents. The solubility parameter of the polymer is expected to be very close to the solubility parameter of the solvent in which it dissolves or swells most.

The monomer 6-methylthio-9-(N-vinylcabamoyl) purine is soluble in many commonly available laboratory solvents. For details refer to Table #20.

Further work on separation of monomer was focused on the chromatographic technique. Sweetman & Nyhan[22] had reported chromatographic separation of purines and pyrimidines through Sephadex G-10 column. The recovery was almost 99% with excellent reproducibility.

Silica gel was selected as the absorbent and chloroform as eluent because of its ability to make the column transparent. During the elution, separation bands could not be detected visually nor by UV fluorescence. Different eluents were tried one after another and evaporated to recover any eluted fraction present. It was found that the pure monomer elutes as a first fraction with chloroform, however, the end cut coming with chloroform shows some presence of impurity. Acetone elutes the third fraction. A trace quantity of fourth fraction is eluted with methylacetate. Dimethylsulfoxide (DMSO) elutes the fifth yellow fraction and renders the column colorless. The yellow fraction (V) could not be recovered from DMSO. The others were recovered by evaporating the solvent under vacuum.

Three partially purified monomer samples were run in a 20 mm dia, 600 mm long glass column. The details are covered in the next section.

TABLE #20

SOLUBILITY OF MONOMER[34]

#	Solvent	Solubility Parameter hildebrands	Remarks	Hydrogen -Bonding Group
1.	Pentane(n)	7.7	Insoluble	p
2.	Heptane(n)	7.4	Insoluble	p
3.	Cyclo-hexane	8.2	Insoluble	p
4.	Amyl acetate	8.3	Soluble	m
5.	Carbon tetrachloride	8.6	Soluble	p
6.	Xylene	8.8	Soluble	p
7.	Toluene	8.9	Soluble	p
8.	Ethyl acetate	9.1	Soluble	m
9.	Tetrahydrofuran	9.1	Soluble	m
10.	Benzene	9.2	Soluble	p
11.	Chloroform	9.3	Soluble	p
12.	Methyl ethyl ketone	9.3	Soluble	m
13.	Monochlorobenzene	9.5	Soluble	p
14.	Methylene chloride	9.7	Soluble	p
15.	Acetone	9.9	Soluble	m
16.	Cyclohexanone	9.9	Soluble	m
17.	Dioxane	10.0	Soluble	m
18.	Nitrobenzene	10.0	Soluble	p
19.	Aniline	10.3	Soluble	s
20.	Pyridine	10.7	Soluble	s

Continued

21.	Dimethylsulfoxide	12.0	Soluble	m
22.	Dimethylformamide	12.1	Soluble	m
23.	Benzyl alcohol	12.1	Soluble	s
24.	Ethyl alcohol	12.7	Insoluble	s
25.	Methanol	14.5	Insoluble	s
26.	Water	23.4	Insoluble	s

p = Poor

m = Moderate

s = Strong

Chromatographic Separation

Materials

1.	Silica gel.	0.2/0.5 mm. E. Merck.
2.	Chloroform	Baker, Fisher certified
3.	Acetone	Baker, ACS grade
4.	Methyl acetate	MCB (Chromatographic quality)
5.	Dimethylsulfoxide	Malkinckrodt (AR)
6.	Column	20 mm dia., 600 mm long glass column with sintered disc and a valve at the bottom

Column Preparation

A slurry of silica gel was made in chloroform and charged into the column containing chloroform. The addition was in batches, while the tube was kept vibrating. The packing was compact and free from air bubbles. Partially purified monomer (2.0 g) was dissolved in 45.0 ml chloroform and charged at the top of the column. A typical elution program is presented in Table #21.

The column remains transparent with chloroform but becomes opaque with acetone and methyl acetate. The top region takes a yellow stain which begins to elute with DMSO. Fraction (I) is pure monomer, while fraction (II) is monomer with some contamination. Fraction (III) is insoluble in chloroform/acetone mixture. Fraction (IV) is present only in trace quantities. Fraction (V) is a yellow material which could not be separated from DMSO. The yield of the fractions is reported in Table #22.

TABLE #21

ELUTION PROGRAM

Eluent				Fraction	
Cut #	Volume ml	Time min.	Solvent	Weight g	#
1.	125	30	$CHCl_3$	nil	-
2.	125	15	$CHCl_3$	nil	-
3.	125	15	$CHCl_3$	0.114	I
4.	125	12	$CHCl_3$	0.535	I
5.	125	13	$CHCl_3$	0.453	I
6.	125	15	$CHCl_3$	0.244	II
7.	125	13	$CHCl_3$	0.013	II
8.	125	14	$CHCl_3$	0.005	II
9.	125	15	$CHCl_3$/Acetone	0.005	III
10.	125	12	Acetone	0.385	III
11.	125	15	Acetone	0.097	III
12.	125	10	Acetone/MeAc.	nil	
13.	125	15	MeAc	Traces	IV
14.	125	12	MeAc	nil	
15.	125	17	MeAc/DMSO	Yellow solu-	V
16.	125	15	MeAc/DMSO	tion	V

Symbols:

1. CH_3Cl Chloroform
2. MeAc Methyl acetate
3. DMSO Dimethyl sulfoxide

TABLE #22

YIELD OF THE FRACTIONS

Fraction #	RUN # 1 Qty. g	1 %	2 Qty. g	2 %	3 Qty. g	3 %	Average Qty. g	Average %
Monomer	2.00	-	2.00	-	2.00	-	2.00	-
I	1.35	67.0	1.147	57.3	1.428	71.4	1.30	65.2
II	0.02	-	0.277	13.8	Trace	-	0.10	4.9
III	0.35	17.5	0.487	24.4	0.420	21.0	0.42	21.0
IV	Trace	-	Trace	-	Trace	-	Trace	-
V	----------Not Recoverable----------							

The physical characteristics of each fraction is presented in subsequent Tables.

TABLE #23

PHYSICAL CHARACTERISTICS OF FRACTION #I

Particulars	RUN # 1	2	3
Appearance	White fine crystals.		
Soluble in	$CHCl_3$, Acetone, CH_2Cl_2 Methyl ethyl ketone & CCl_4		
Insoluble in	n-Hexane and n-Pentane.		
Melting Range			
1. Shows signs of melting at °C	166.5	169.0	169.0
2. Effervascence at °C	167.5	170.0	169.0
3. Colorless transparent at °C	169.0	171.5	170.0

The fraction is very heat sensitive and decomposes at 169°C. The IR spectrum is consistant with the structure of the monomer. The purity is of very high order as evident from the elemental analysis report presented in Table #24.

TABLE #24

ELEMENTAL ANALYSIS OF FRACTION #I

Elements	Theoretical %	Actual %	Difference
1. Carbon	45.96	45.99	+0.03
2. Hydrogen	3.83	3.71	-0.12
3. Nitrogen	29.79	29.98	+0.19
4. Sulfur	13.62	13.35	-0.27
5. Oxygen	6.81	6.97	+-.16

TABLE #25

PHYSICAL CHARACTERISTICS OF FRACTION #II

Particulars	RUN # 1	2	3
Appearance	----White fine crystals---		
Soluble in	$CHCl_3$, Acetone, CH_2Cl_2, MEK and CCl_4		
Insoluble in	n-Hexane and n-Pentane		
Melting Range:			
1. Shows signs of melting at	---------165°C----------		
2. Effervascence at	-------167/168°C----------		
3. Colorless transparent liquid at	---------169°C----------		

The fraction seems to be impure monomer, and is similar to the material recrystallized from the chloroform/hexane mixture (Tables 17 & 18).

Fraction #III

This fraction elutes with acetone but becomes turbid in a mixture of acetone/chloroform. The turbidity disappears when the acetone ratio in the mixture increases. The material is very heat sensitive and effervesces at temperatures below 120°C. The IR of this fraction is similar to that of the resinous material (Figure #7).

Fraction #IV

This fraction elutes with methyl acetate. It is a yellow material present only in trace quantities.

Fraction #V

This fraction is held at the top of the column and elutes only with DMSO as a yellow solution. It could not be recovered either by precipitation or evaporation.

The Procedure for the Polymerization of the Monomer

Purified monomer obtained by recrystallization and chromatographic separation was polymerized by free radical mechanism in benzene. A solution of benzene containing monomer and AIBN, 2,2'-azobis-(2-methyl propionitrile), was charged into a 150-ml three-necked flask. It was fitted with a magnetic stirrer, a reflux condenser and a thermometer. The reactants and the reaction flask were deoxygenated by bubbling nitrogen gas for fifteen minutes. The solution was heated on a glycerin bath to attain reflux. The polymerization was continued for several hours at the reflux temperature of benzene. Details of the polymerization are recorded in Table #26 below.

TABLE #26

FREE RADICAL POLYMERIZATION OF MONOMER

Particulars	Molecular Weight	BATCH # 1	BATCH # 2
1. Monomer	235.0	0.47g	0.47g
2. AIBN	164.0	3.3mg	6.6mg
3. Benzene	-	100 ml	100ml
4. Mole % of AIBN on Monomer	-	1.0%	2.0%
5. Polymerization Time	-	15 hrs.	24 hrs. (I Sample) 48 hrs. (II Sample)
6. Polymerization Temperature	-	80°C	80°C

Recrystallized monomer was used in Batch #1 and chromatographically separated monomer in Batch #2. The AIBN was freshly crystallized from methanol. Benzene was first dried over calcium chloride, refluxed with sodium metal and distilled. Two samples were obtained from Batch #2 after polymerizing for 24 and 48 hours, no improvement in yield was noted.

Recovery of the Polymer

No change in appearance in the reaction mixture was noted during or after the polymerization. To effect separation of the polymer formed, 85% of the benzene charged was evaporated. No separation in the concentrated solution was observed, indicating the solubility of the polymer to be higher than that of the monomer. A white solid separates out on chilling in an ice bath, but redissolves on warming to room temperature.

To the 15.0 ml concentrated reaction mixture from Batch #1 was added 75.0 ml methanol, no separation was noted. A white solid (P-11) precipitated out at $0^{o}C$ which did not redissolve at room temperature. It was decanted, washed with methanol and dried under vacuum. The melting range and IR suggests it to be

impure monomer. The clear filtrate was evaporated and dried to give a white powder. The IR suggests it to be a polymer. A schematic presentation of the process is given below.

Conc. Batch #1 15.0 ml
Methanol 75.0 ml
-- Reaction Mixture (P-I)

(PI)

White Ppt. (P-11)

Clear Solution evaporated, gives white powder (P-12)

Physical properties are presented in Tables #27 & 28. The IR spectrum of fraction P-12 show a reduction in C=C peak intensity and appears to be polymeric.

TABLE #27

PHYSICAL CHARACTERISTICS OF FRACTION P-11

1.	Signs of melting	166°C
2.	Effervascence	167°C
3.	Transluscent speck	172°C
4.	No change	205°C
5.	Reddish liquid	210°C

The material closely resembles crude monomer.

TABLE #28

PHYSICAL CHARACTERISTICS OF FRACTION P-12

1.	White solid	188°C
2.	Solid changes to yellowish	189°C
3.	Solid light brown opaque	205°C
4.	Solid fast change to brown	206°C
5.	Opaque dark brown & foaming	210°C
6.	IR-Spectra	Figure #6

Infrared Analysis Report

The synthesized products, 6-methylthiopurine, the monomer 6-methylthio-9-(N-vinylcarbamoyl)purine and the polymer were scanned by infrared, to confirm the structures, using KBr pellets in a IR-12 Beckman Spectrophotometer in the frequency range 4000-650 cm^{-1}.

I. 6-Mercatopurine

The IR-Spectra of 6-mercaptopurine is presented in Figure #3 and its assignable peaks in Table #29.

II. 6-Methylthiopurine

The spectral details of 6-methylthiopurine are presented in Figure #4 and Table #30.

III. 6-Methylthio-9-(N-Vinylcarbamoyl)purine

Figure #5 and Table #31 present the spectral details of the monomer. Note the disappearance of the ten moderate to weak bands caused due to the N-H at 9-position[21] because of the vinyl-isocyanate substitution.

The second point to note is that the bands due to -NH(carbamate), C=C(vinyl) and C-H(ring) overlap at 3100 cm^{-1} and form a sharp band. When the C=C (vinyl) band disappears (due to polymerization) this band becomes broad.

IV. Polymer

The polymer is probably impure. The vinyl C=C bands at 835, 860, 920, 3040 and 3100 cm^{-1} have reduced almost 90% leaving residual bands. The most intriguing and puzzling feature is the reduction of the carbonyl (C=O) bands at 1655 and 1740 cm^{-1}. No explanation is known for this. The details may be noted in Figure #6.

V. Resinous Material

Figure 7 shows the IR spectrum of the resinous material separated from the crude monomer. This spectrum is similar to that of the polymer (Figure 6).

TABLE #29

IR-BANDS OF 6-MERCAPTOPURINE

#	GROUPS	Bands Frequency cm^{-1}	Intensity	Ref:
1.	S-H	670	W/M	35
2.	Unassigned	870	S	--
3.	Purine	930	M	35
4.	C-H deformation	1010	M/W	35
5.	Unassigned	1110	M/W	--
6.	Unassigned	1200	S/M	--
7.	C-N Vibrations	1210	W	19
8.	S-H	1330	S/M	36
9.	C-H, C=C (ring)	1405	S	19 & 35
10.	C-H bending	1435	S	19
11.	C-H (also S-H)	1460	B	19
12.	Purine	1555	M	35
13.	C=N motions	1565	M	19
14.	N-H at 9-position about ten moderate to weak bands	2540 to 3070	M to W	21
15.	C=C (ring)	3100	M	35
16.	Unassigned	3450	B	--

Symbols

S - Strong
M - Moderate
W - Weak
B - Broad

S/M - Strong to moderate
M/S - Moderate to strong
M/W - Moderate to weak
W/M - Weak to moderate

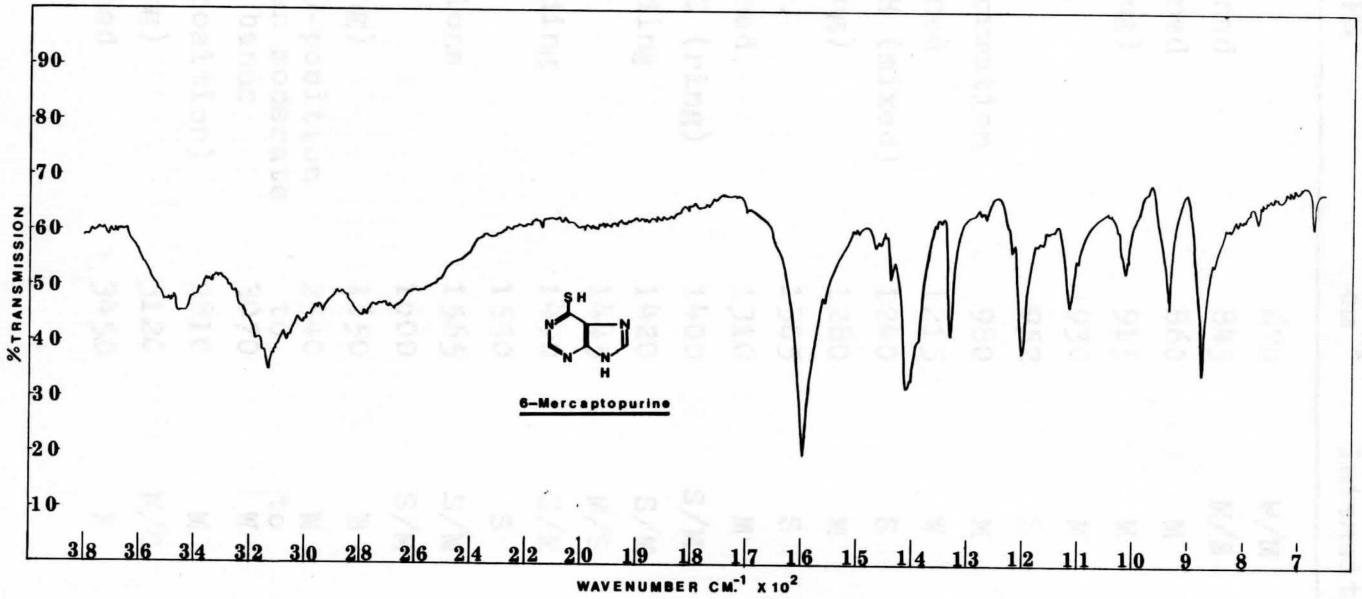


FIGURE #3

IR-Spectra of 6-Mercaptopurine

TABLE #30

IR-BANDS OF 6-METHYLTHIOPURINE

#	GROUPS	Band Frequency cm^{-1}	Intensity	Ref:
1.	S-CH_3	670	W/M	35
2.	Unassigned	845	M/B	--
3.	Unassigned	860	M	36
4.	C=C (ring)	915	M	19
5.	Purine	930	M	35 & 36
6.	Purine	952	S	35
7.	C-H deformation	980	M	35
8.	Unassigned	1215	W	--
9.	C-N, C-H (mixed)	1240	S	19 & 35
10.	C=C (ring)	1280	M	19
11.	S-CH_3	1305	S	36
12.	Unassigned	1310	M	--
13.	C-H, C=C (ring)	1400	S/M	19 & 35
14.	C-H bending	1420	S/M	19
15.	S-CH_3	1445	M/S	36
16.	C-H bending	1490	S/M	19
17.	Purine	1550	S	35
18.	C=N motions	1565	S/M	19
19.	Purine	1600	S/M	35
20.	C=C (ring)	1650	B	19 & 36
21.	N-H of 9-position about ten moderate to weak bands	2540 to 3070	M to W	21
22.	N-H (9-position)	2810	M	21
23.	C=C (ring)	3120	M/S	35
24.	Unassigned	3450	B	--

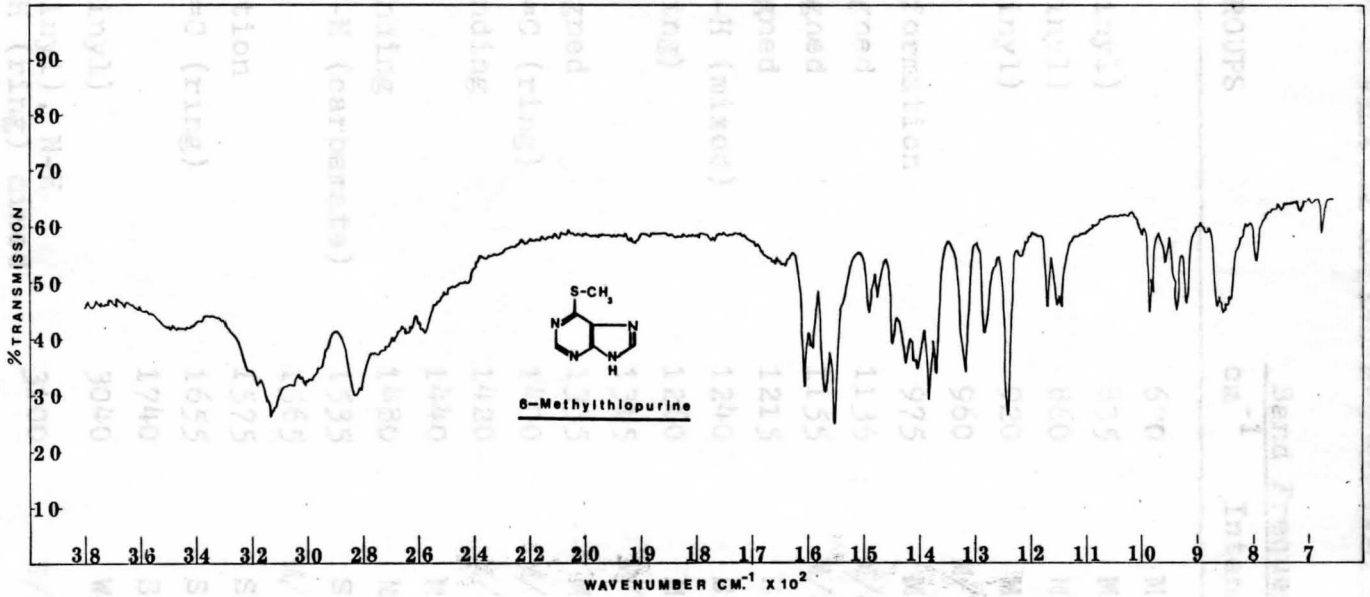


FIGURE #4

IR-Spectra of 6-Methylthiopurine

TABLE #31

IR-BANDS OF THE MONOMER, 6-METHYLTHIO-9-(N-VINYLCARBAMOYL) PURINE

#	GROUPS	Band Frequency cm^{-1}	Intensity	Ref:
1.	$S-CH_3$	670	M	35
2.	C=C (vinyl)	835	M	36
3.	C=C (vinyl)	860	M	36
4.	C=C (vinyl)	920	M	36
5.	Purine	960	M/W	35
6.	C-H deformation	975	M	35
7.	Unassigned	1135	M/S	--
8.	Unassigned	1155	M/S	--
9.	Unassigned	1215	S	--
10.	C-N, C-H (mixed)	1240	S	19 & 35
11.	C=C (ring)	1260	M	35
12.	$S-CH_3$	1315	M/W	36
13.	Unassigned	1335	M	--
14.	C-H, C=C (ring)	1400	M/W	19 & 35
15.	C-H bending	1420	M/W	19
16.	$S-CH_3$	1440	M	36
17.	C-H bending	1480	M	19
18.	C=N, N-H (carbamate)	1535	S	19 & 35
19.	Purine	1565	M/S	35
20.	C=N motion	1575	S	19
21.	C=O, C=C (ring)	1655	S	36 & 19
22.	C=O	1740	S	19 & 33
23.	C=C (vinyl)	3040	W	35
24.	C=C (vinyl), N-H and C-H (ring) mixed	3100	S/M	35, 19 & 36
25.	N-H (carbamate)	3250	M	35

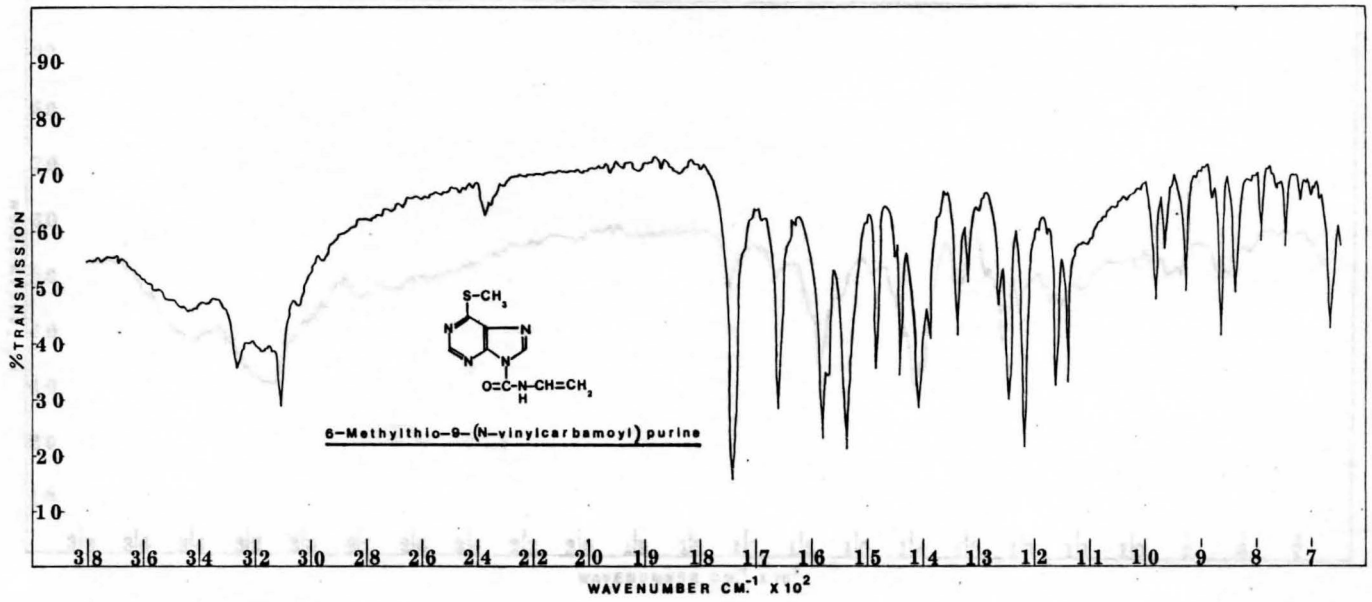


FIGURE #5

IR-Spectra of 6-Methylthio-9-(N-Vinylcarbamoyl) Purine

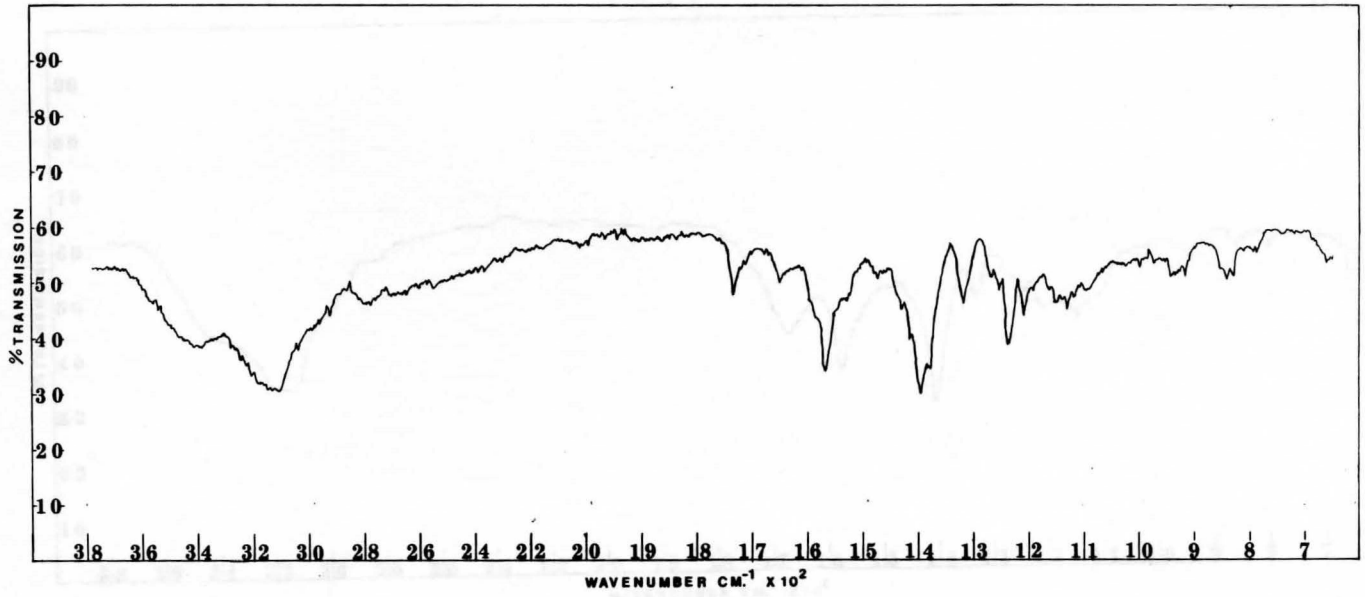


FIGURE #6

IR-Spectra of Polymerized 6-Methylthio-9-(N-Vinylcarbamoyl) Purine

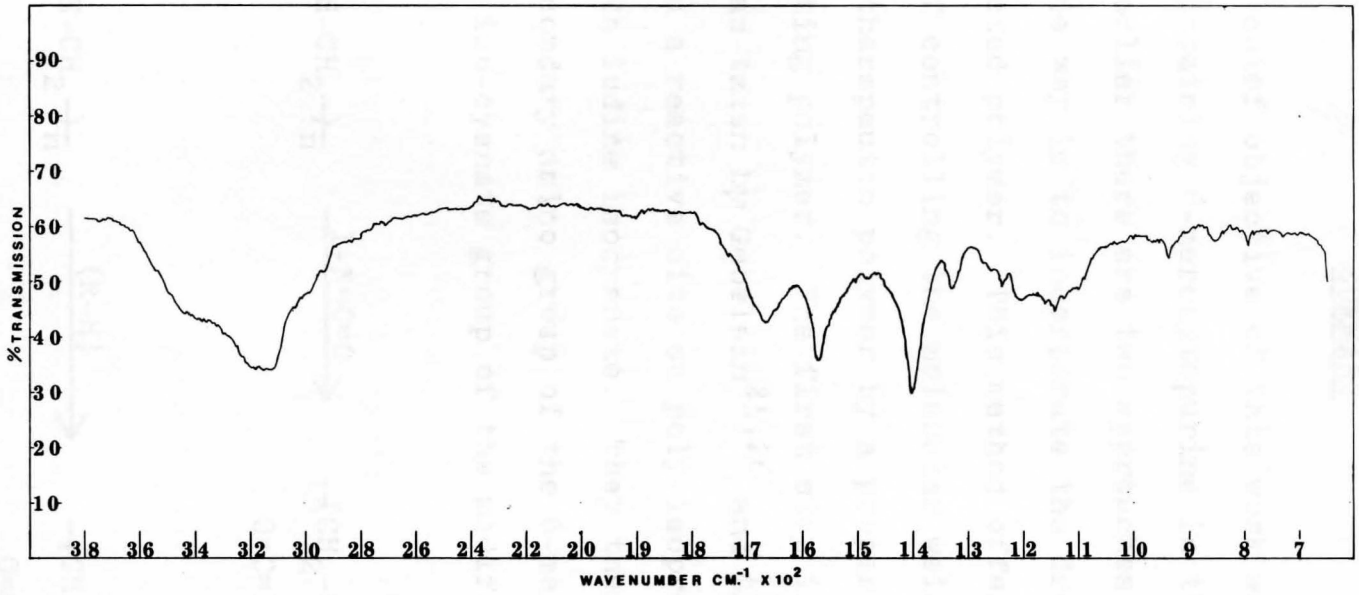


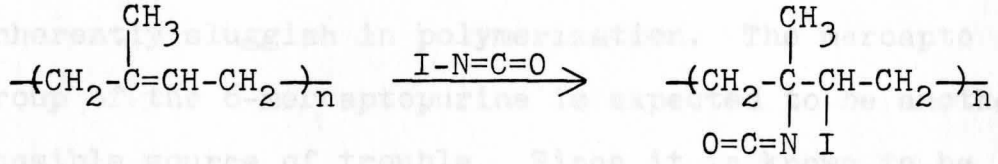
FIGURE #7

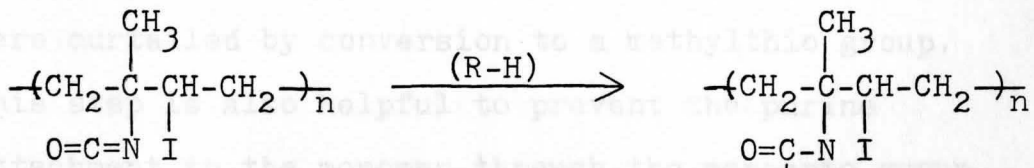
IR-Spectra of Brown Resinous Impurity From Crude Monomer

CHAPTER V

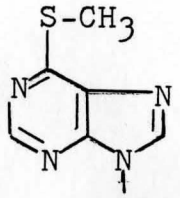
SUMMARY

The chief objective of this work was to produce a polymer containing 6-mercaptopurine in the side chain. As stated earlier there are two approaches to this problem. One way is to incorporate the drug directly onto a selected polymer. This method offers the advantage of controlling the molecular weight of the final chemotherapeutic polymer by a proper selection of the starting polymer. The first step in this direction was taken by Gebelein[25,26] and Glowacky[27]. They created a reactive cite on poly-isoprene by reaction with iodine isocyanate. They then hoped to link the secondary amino group of the 6-mercaptopurine through the iso-cyanate group of the modified polymer.





where R=



His work did not proceed further due to limitation of the solubility of the drug and the modified polymer. No common solvent could be found to serve as reaction media.

So it became inevitable to adopt the second line of approach. In this method the drug is incorporated onto a polymerizable monomer and later polymerized to the desired degree of polymerization. This method gives a wider latitude for modification but poses serious problems in purification and control of degree of polymerization. Glowacky[27] attempted to incorporate 6-methylthiopurine in acrylic and allylic monomers. The work did not proceed beyond modification of the monomer as the first could not be purified and the second failed to polymerize. The failure of the attempts was due to the nature of the monomers selected. The acrylic monomer (acryloyl chloride) has high tendency to homo-polymerize while the allylic monomer is inherently sluggish in polymerization. The mercapto group of the 6-mercaptopurine is expected to be another possible source of trouble. Since it is known to be a chain transfering agent, its inhibitory characteristics were curtailed by conversion to a methylthio group. This step is also helpful to prevent the purine attachment to the monomer through the mercapto group.

This work dealt with the modification of vinyl-isocyanate with 6-methylthiopurine in the presence of triethylamine. An efficient and quick chromatographic method for the separation of the pure monomeric 6-methylthio-9-(N-vinylcarbamoyl)purine was developed. The general method of recrystallization was effective only at very low temperatures (-80°C), the yields were low (27%) and purity inadequate. Chromatographic separation on a silica gel column provided high yield (71%) and the purity close to the theoretical elemental analysis values. IR-Spectroscopy was the other chief tool to establish the structure. The C=C (vinyl), C=O (carbonyl), -NH-C=O (amide) and the other purine bands matched with the literature reported values in related compounds. The substitution of the 9-H in purine is well established. This monomer is thermally stable. Prolonged heating at 100°C does not bring any change in the IR pattern, but it undergoes a very quick decomposition close to its melting point (169°C). The monomer hydrolyzes when heated with water, yielding 6-methylthiopurine. It is soluble in many laboratory solvents having a solubility parameter in the range 8.3-12.1 hildebrands.

The monomer is polymerizable by a free radical mechanism with 2,2'-azobis-(2-methyl propionitrile) in benzene at 80°C. The polymer is more soluble in benzene and benzene/methanol mixture than the monomer.

Hence, the monomer was separated by precipitation, and the polymer was recovered by the evaporation of the filtrate. The crude polymer thus obtained was checked for the polymeric characteristics. A distinct change in IR occurred in comparison to that of the monomer. The C=C (vinyl) bands almost disappeared which is a positive proof of the presence of the polymer. Its melting point was elevated, and remained unchanged until 188°C, then turned yellow to dark brown and finally foamed at 210°C.

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