Unusual Dihydrazone Formation in the Fischer-Indole Cyclization. The Synthesis of Novel Nonclassical Annulated 2,4-Diamino-pyrrolo[2,3-*d*]pyrimidine Antifolates [1]

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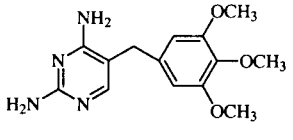
Received December 12, 1998

The synthesis of seven novel tetracyclic 2,4-diaminopyrrolo[2,3-*d*]pyrimidines as conformationally restricted nonclassical antifolates was achieved *via* an unusual Fischer-indole cyclization of dihydrazones. An attempted synthesis of 2,4-diamino-6-hydrazinopyrimidine afforded 2-amino-4,6-dihydrazinopyrimidine which when reacted with appropriate ketones gave the dihydrazones. The dihydrazones in turn under Fischer-indole cyclization conditions afforded target conformationally restricted tetracyclic products.

J. Heterocyclic Chem., **36**, 441 (1999).

Dihydrofolate reductase is an essential enzyme involved in the folate metabolic pathway which catalyzes the NADPH-linked reduction of dihydrofolate to tetrahydrofolate, and couples with thymidylate synthase in the biosynthesis of thymidylate from 2'-deoxyuridylate [2]. Inhibition of dihydrofolate reductase leads to an indirect inhibition of DNA synthesis by depletion of the cellular tetrahydrofolate pool and results in cell death. Thus, dihydrofolate reductase inhibitors have been employed clinically as antimicrobials, antineoplastics and antiprotozoan agents [3]. Trimethoprim (**1**) is a first line drug for the treatment of *Pneumocystis carinii* pneumonia, an opportunistic infection in patients with acquired immunodeficiency syndrome (AIDS) [4]. As part of a project aimed at developing potential dihydrofolate reductase inhibitors, we report here the synthesis of novel tetracyclic 2,4-diaminopyrrolo[2,3-*d*]pyrimidines **2-8** (Scheme 1) as conformationally restricted analogues of trimethoprim.

Scheme 1



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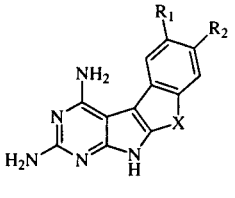
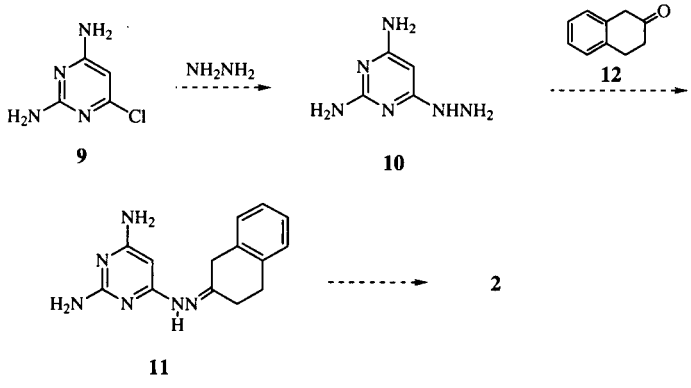


2, X = -CH_2CH_2-, $R_1 = R_2 = H$
3, X = -CH_2CH_2-, $R_1 = OCH_3$, $R_2 = H$
4, X = -CH_2CH_2-, $R_1 = H$, $R_2 = OCH_3$
5, X = -CH_2CH_2-, $R_1 = R_2 = OCH_3$
6, X = -CH_2-, $R_1 = R_2 = H$
7, X = -CH=CH-, $R_1 = R_2 = H$
8, X = -CH=CH-, $R_1 = R_2 = OCH_3$

The synthesis of the target compounds **2-6** using the Fischer-indole cyclization was envisioned *via* a three step sequence (Scheme 2) involving the synthesis of the pyrimidin-6-yl hydrazine **10**, followed by the formation of the hydrazones **11** with the appropriate ketones and the

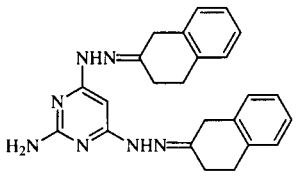
Scheme 2
Attempted Synthesis of **2** *via* the Fischer-indole cyclization



9 **10** **11** **12**

Fischer-indole cyclization to afford **2-6**. Thus, commercially available 4-chloro-2,6-diaminopyrimidine (**9**) was refluxed with excess hydrazine hydrate in *n*-butanol for 6 hours. A white precipitate was obtained which was presumed to be the hydrazine **10**. This was directly utilized for hydrazone formation with 2-tetralone (**12**) in ethanol at reflux until the hydrazine precipitate disappeared. The resulting product was, however, identified as the bis-hydrazone **13** (Scheme 3) by its ^1H and ^{13}C nmr, ms and elemental analysis rather than the expected monohydrazone **11**. The ^1H nmr spectrum showed a single amino signal at 5.55 ppm. More importantly, the aromatic protons as well as the methylene protons anticipated for **11** integrated for twice as many protons as expected, compared with the H5 proton (6.11 ppm) of the pyrimidine ring. This suggested that two equivalents of the ketone **12** had reacted with one equivalent of the pyrimidine hydrazine **10**. It was however, unlikely that Schiff base formation of the ketone **12** with the 4-amino group of the pyrimidine was responsible for the absence of the second amino group in the ^1H nmr of the product. Such a monohydrazone-mono-Schiff base compound **16** would exhibit

Scheme 3



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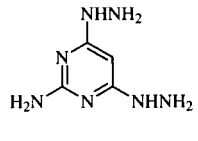
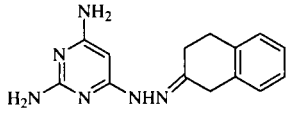


14



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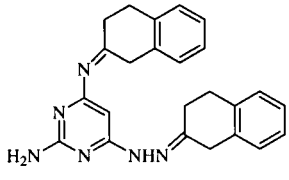

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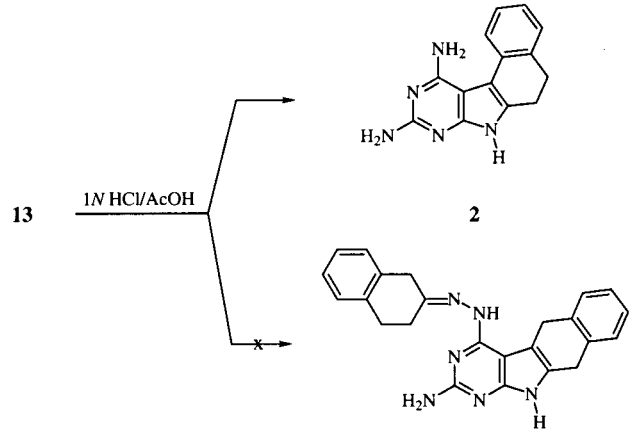
a single proton signal for the hydrazone NH. However, the NH signal at 8.79 ppm integrated for two protons, suggesting the formation of a dihydrazone as depicted in structure **13**.

Additional evidence for the structure of the dihydrazone **13** was obtained from its ^{13}C nmr and mass spectrum. The mass spectrum of **13** indicated a molecular ion mass at *m/z* 411, which was consistent with the molecular weight of the dihydrazone shown in the structure **13**. The decoupled ^{13}C nmr spectrum revealed thirteen carbon signals, which was one carbon less than that expected from the ^{13}C nmr of the other two possible structures: *i.e.* the monohydrazone **15** and the Schiff base-hydrazone **16**. Thus the ^{13}C nmr of thirteen signals could only arise from a symmetrical dihydrazone structure. In addition, the elemental analysis also corroborated the formation of the dihydrazone **13**.

Having established the structure of **13** as the dihydrazone, it was obvious that the precursor of the dihydrazone must have been the dihydrazine **14**, rather than the expected monohydrazone **10**, and further that **14** rather than **10** must have formed in the reaction of pyrimidine **9** and hydrazine. The acidic or alkaline hydrolysis of 2,4-diaminopyrimidines and 2,4-diamino fused pyrimidines to the corresponding oxo analogues is well documented in the literature [5]. Thus it was highly probable that the displacement of the 4-amino moiety of **9** with hydrazine, a powerful nucleophile, must have occurred during the reaction to afford the dihydrazine **14**.

Although no dihydrazone associated Fischer-indole cyclization has been reported in the literature, it is documented that the direction of cyclization in a Fischer-indole reaction is dependent on the solvent utilized, the nature and amount of catalyst as well as the conditions (thermal *vs.* acid catalysis) of the cyclization procedure [6]. Numerous acidic solvents and Lewis acid catalysts have been used in Fischer-indole cyclization [6]. Saturated hydrogen chloride in acetic acid has been reported [6,7] to improve regioselectivity at the benzylic position in the Fischer-indole cyclization of phenylhydrazone **17**, which afforded the angular structure **18** rather than the linear structure **19** (Scheme 4). Thus, the dihydrazone **13** when heated with commercially available 1*N* hydrogen chloride in acetic acid (Scheme 5) afforded

Scheme 5



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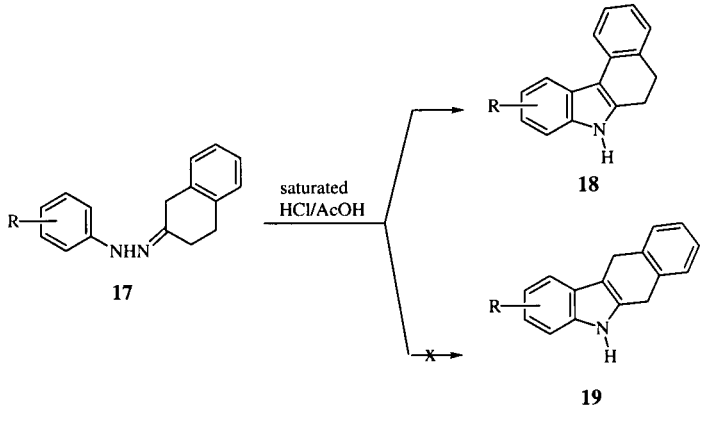
multiple spots on tlc analysis. Column chromatographic separation of the mixture of products afforded fractions with a R_f of 0.44 on tlc (methylene chloride-methanol, 8:1) which were pooled and identified as the cyclized product **2**. The structure of **2** was established by the absence of the pyrimidine H5 signal and the presence of a new pyrrole NH signal at 11.00 ppm in its ^1H nmr spectrum. It was further characterized by the mass spectrum and elemental analysis. This implied that one of the hydrazones was converted back to the amine during the cyclization reaction which affords **2** rather than the expected structure **20.** Compound **20** was the anticipated product from the cyclization of the dihydrazone **13**. This

Scheme 4



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unusual replacement could have occured *via* the displacement of one of the hydrazones of **13** by the ammonia liberated during the Fischer-indole type reaction [8].

Compounds **3-6** were similarly prepared from the corresponding dihydrazones of the appropriate ketones. These analogues were characterized *via* their ^{1}H nmr spectra and elemental analyses. Due to its propensity to aromatize, the ^{1}H nmr of **5** consisted of a mixture of **5** and its aromatized product **8**.

The fully-aromatized compounds **7** and **8** were obtained *via* the oxidation of **2** and **5** respectively with manganese dioxide in acetic acid and dimethyl sulfoxide. The structures of the oxidized products were confirmed by the absence of the methylene signals and the presence of two new aromatic doublets at 7.94 and 8.70 ppm for **7**, and at 7.44 and 7.74 for **8**. This together with the appropriate elemental analyses established the structures of **7** and **8** as shown.

In summary, we report, for the first time, that the dihydrazine of 4-chloro-2,6-diaminopyrimidine when reacted with ketones leads to the dihydrazone, which in an unusual Fischer-indole cyclization affords the Fischer-indole product expected from the monohydrazone.

EXPERIMENTAL

Starting materials used in the synthetic procedures were obtained from Aldrich Chemical Co., Milwaukee, WI. Melting points were determined on a Fisher-Johns melting point apparatus and are uncorrected. The ^{1}H and ^{13}C nmr spectra were recorded on a Bruker WH-300 (300 MHz) spectrometer at 125 MHz in dimethyl-d_6 sulfoxide with tetramethylsilane as the internal standard. The chemical shift values are expressed in δ values (parts per million) relative to tetramethylsilane as an internal standard: s = single, d = doublet, t = triplet, m = multiplet. Mass spectra were recorded on a Varian MATCH-311A mass spectrometer in the electron ionization (EI) mode. Thin-layer chromatography (tlc) was performed on silica gel plates with fluorescent indicator and were visualized with light at 254 and 366 nm. Ratios of solvents are volume/volume. Elemental analyses were performed by Atlantic Microlab, Inc., Norcross, GA. Analytical results indicated by element symbols were within ± 0.4% of the theoretical values. Compounds were dried in an Abderhalden drying apparatus, over phosphorus pentoxide for a period of 24 hours, at ≤0.20 mm Hg (with heating over ethanol) prior to submission for elemental analysis. Fractional amounts of solvents could not be removed despite drying under vaccum for 24 hours and were confirmed, where possible, in the ^{1}H nmr.

2-Amino-4,6-di(2'-tetralenyldienehydrazino)pyrimidine (**13**).

A mixture of 4-chloro-2,6-diaminopyrimidine (1.45 g, 10.0 mmoles) and hydrazine hydrate (1.2 ml, 20.0 mmoles) (55% aqueous solution) in *n*-butanol (20 ml) was refluxed for 6 hours. The mixture was then cooled to room temperature and triturated with 20 ml of methanol. A white solid was obtained and collected by filtration, washed with methanol and air-dried to afford the crude hydrazine **14** (1.0 g), which was not further characterized but used directly in the next step.

A mixture of the crude hydrazine (0.70 g, 4.50 mmoles) and 2-tetralone (1.20 ml, 9.00 mmole) in 80 ml of ethanol was refluxed in the dark for 3 hours. After cooling, the resulting precipitate was filtered, washed with water and dried *in vacuo* to afford 1.68 g (90.5%) of **13** as a yellow solid, mp 190° dec; ^{1}H nmr: δ 2.57 (t, 4H), 2.87 (t, 4H), 3.59 (s, 4H), 5.55 (s, 2H, exchangeable with deuterium oxide), 6.11 (t, 1H), 7.27-7.15 (m, 8H), 8.79 (s, 2H, exchangeable with deuterium oxide); ^{13}C nmr: δ 25.6, 27.2, 37.8, 74.0, 126.3, 126.4, 127.0, 127.2, 136.0, 138.4, 151.1, 162.3, 163.5; ms: m/z 411 (M^+).

Anal. Calcd. for $C_{24}H_{25}N_7$: C, 70.07; H, 6.08; N, 23.84. Found: C, 70.03; H, 6.17; N, 23.84.

9,11-Diamino-5,6-dihydro-7*H*-benzo[*e*]pyrimido[4,5-*b*]indole (**2**).

A suspension of **13** (1.00 g, 2.43 mmoles) in 30 ml of 1*N* hydrogen chloride in acetic acid was heated with stirring in a preheated oil bath (100-105°) for 4 hours. The resulting dark solution was cooled and kept at room temperature overnight. To this solution was added a small amount of silica gel and the solvent evaporated under vacuo at 75°. The residue was loaded onto a dry silica gel column and eluted first with 400 ml of methylene chloride-methanol (96:4), followed by 500 ml of methylene chloride-methanol-ammonium hydroxide (32:4:0.5). The fractions containing the product (tlc, R_f = 0.44, methylene chloride-methanol, 8:1) were pooled, concentrated *in vacuo*, and crystallized from acetic acid to yield 0.20 g (33%) of **2** as white crystals: mp 253-255°; ^{1}H nmr: δ 2.65 (t, 2H), 2.87 (t, 2H), 5.51 (br s, 2H, exchangeable with deuterium oxide), 5.81 (br s, 2H, exchangeable with deuterium oxide), 6.99 (t, 1H), 7.22 (m, 2H), 7.51 (d, 1H), 11.00 (s, 1H) (exchangeable with deuterium oxide); ms: m/z 251 (M^+).

Anal. Calcd. for $C_{14}H_{13}N_5 \cdot 2.8CH_3COOH$: C, 56.13; H, 5.82; N, 16.70. Found: C, 55.99; H, 5.88; N, 16.76.

9,11-Diamino-5,6-dihydro-7*H*-3-methoxybenzo[*e*]pyrimido-[4,5-*b*]indole (**3**).

Using a procedure similar to that described for **13** and **2**, compound **3** was prepared from crude **14** (0.22 g, 1.42 mmoles) and 6-methoxy-2-tetralone (0.50 g, 2.84 mmoles) and recrystallized from acetic acid (0.08 g, 20% in 2 steps), mp 294-298°; tlc, R_f = 0.38 (methylene chloride-methanol, 8:1); ^{1}H nmr: δ 2.63 (t, 2H), 2.85 (t, 2H), 5.50 (br s, 2H, exchangeable with deuterium oxide), 5.80 (br s, 2H, exchangeable with deuterium oxide), 6.81 (d, J = 8.36 Hz, 1H), 6.85 (s, 1H), 7.43 (d, J = 8.36 Hz, 1H), 10.91 (br s, 1H, exchangeable with deuterium oxide); ms: m/z 281 (M^+).

Anal. Calcd. for $C_{15}H_{15}N_5O$: C, 64.04; H, 5.37; N, 24.89. Found: C, 64.18; H, 5.39; N, 24.93.

9,11-Diamino-5,6-dihydro-7*H*-2-methoxybenzo[*e*]pyrimido-[4,5-*b*]indole (**4**).

Using a procedure similar to that described for **13** and **2**, compound **4** was prepared from crude **14** (0.22 g, 1.42 mmoles) and 7-methoxy-2-tetralone (0.50 g, 2.84 mmoles) and recrystallized from acetic acid (0.05 g, 13%), mp 230° dec; tlc, R_f = 0.44 (methylene chloride-methanol, 8:1); ^{1}H nmr: δ 2.63 (t, 2H), 2.80 (t, 2H), 5.55 (br s, 2H, exchangeable with deuterium oxide), 5.87 (br s, 2H, exchangeable with deuterium oxide), 6.55 (dd, J = 8.17, 2.50 Hz, 1H), 7.10 (d, J = 8.17 Hz, 1H), 7.15 (d, J = 2.50 Hz, 1H), 11.02 (br s, 1H, exchangeable with deuterium oxide); ms: m/z 281 (M^+).

Anal. Calcd. for $C_{15}H_{15}N_5O \cdot 0.8CH_3COOH$: C, 60.54; H, 5.57; N, 21.26. Found: C, 60.30; H, 5.54; N, 21.12.

2,4-Diamino-9*H*,10*H*-indeno[1',2':4,5]pyrrolo[2,3-*d*]pyrimidine (**6**).

Using a procedure similar to that described for **13** and **2**, compound **6** was prepared from crude **14** (0.45 g, 2.90 mmoles) and 2-indanone (0.43 g, 3.20 mmoles) and recrystallized from acetic acid (0.05 g, 0.7%), mp 250° dec; tlc, R_f = 0.2 (methylene chloride-methanol, 8:1); ^{1}H nmr: δ 3.62 (s, 2H), 5.58 (br s, 2H, exchangeable with deuterium oxide), 6.01 (br s, 2H, exchangeable with deuterium oxide), 7.00 (t, 1H), 7.21 (t, 1H), 7.38 (d, *J* = 7.26 Hz, 1H), 7.65 (d, *J* = 7.47 Hz, 1H), 11.02 (br s, 1H, exchangeable with deuterium oxide).

Anal. Calcd. for $C_{13}H_{11}N_5$•1.0CH_3COOH: C, 60.55; H, 5.04; N, 23.54. Found: C, 60.32; H, 5.10; N, 23.56.

9,11-Diamino-7*H*-benzo[*e*]pyrimido[4,5-*b*]indole (**7**).

To a preheated (110-120°) solution of **2** (0.05 g, 0.2 mmole) in glacial acetic acid (2 ml), was added an excess of manganese dioxide (0.20 g). The mixture was stirred at 110-120° for 5 minutes and filtered to remove the manganese dioxide. On cooling, a precipitate was obtained which was collected by filtration, washed with acetic acid, ethyl ether, and dried *in vacuo* to yield an off-white solid **7** (0.04 g, 81%), mp 280° dec; tlc, R_f = 0.35 (methylene chloride-methanol, 8:1); ^{1}H nmr: δ 5.95 (s, 2H, exchangeable with deuterium oxide), 6.33 (s, 2H, exchangeable with deuterium oxide), 7.38 (t, 1H), 7.54 (m, 2H), 7.71 (d, *J* = 8.64 Hz, 1H), 7.94 (d, *J* = 8.22 Hz, 1H), 8.70 (d, 1H), 11.56 (s, 1H, exchangeable with deuterium oxide); ms: m/z 249 (M^+).

Anal. Calcd. for $C_{14}H_{11}N_5$: C, 67.39; H, 4.41; N, 28.08. Found: C, 67.51; H, 4.64; N, 27.80.

9,11-Diamino-5,6-dihydro-7*H*-2,3-dimethoxybenzo[*e*]pyrimido[4,5-*b*]indole (**5**).

Using a procedure similar to that described for **13** and **2**, compound **5** was prepared from crude **14** (0.10 g, 0.63 mmole) and 6,7-dimethoxy-2-tetralone (0.26 g, 1.26 mmoles). During the cyclization, a precipitate was formed from the reddish-brown solution. After filtration, an off-white precipitate was collected (0.08 g, 41%), mp 300° dec; tlc, R_f = 0.3 (methylene chloride-methanol, 8:1); ^{1}H nmr: (the solution turns a yellow color and indicates a 3:2 mixture of **5** and its oxidized product **8**): δ for **5**: 2.66 (t, 2H), 2.85 (t, 2H), 3.76 (s, 3H), 3.80 (s, 3H), 6.95 (s, 1H), 6.99 (s, 1H), 7.25 (br s, 2H, exchangeable with deuterium oxide), 8.12 (br s, 2H, exchangeable with deuterium oxide), 12.00 (br s, 1H, exchangeable with deuterium oxide); δ for **8**: 3.90 (s, 3H), 3.98 (s, 3H), 7.44 (d, 1H), 7.46 (s, 1H), 7.74-7.75 (m, 2H), 7.60 (br s, 2H, exchangeable with deuterium oxide), 7.82 (br s, 2H, exchangeable with deuterium oxide), 12.41 (br s, 1H, exchangeable with deuterium oxide); ms: m/z 311 (M^+).

9,11-Diamino-2,3-dimethoxy-7*H*-benzo[*e*]pyrimido-[4,5-*b*]indole (**8**).

To a solution of **5** (0.08 g, 0.26 mmole) in dimethyl sulfoxide at 110°, was added manganese dioxide (0.20 g). After the mixture was stirred for 5 minutes at this temperature, the manganese dioxide was filtered. The filtrate was then concentrated *in vacuo* to remove most of solvent, followed by the addition of acetic acid. The resulting precipitate was filtered, washed and dried to yield a gray solid **8** (0.05 g, 63%), mp 285° dec; tlc, R_f = 0.35 (methylene chloride-methanol, 8:1); ^{1}H nmr: δ 3.90 (s, 3H), 3.98 (s, 3H), 7.44 (d, *J* = 8.50 Hz, 1H), 7.46 (s, 1H), 7.49 (br s, 2H, exchangeable with deuterium oxide), 7.74 (d, *J* = 8.50 Hz, 1H), 7.75 (s, 1H), 8.00 (br s, 2H, exchangeable with deuterium oxide), 12.38 (br s, 2H, exchangeable with deuterium oxide); ms: m/z 309 (M^+).

Anal. Calcd for $C_{16}H_{15}N_5O_2$•0.2DMSO•1.0HCl: C, 54.50; H, 4.80; N, 19.38. Found: C, 54.73; H, 4.90; N, 19.16.

Acknowledgments.

This work was supported in part by grants from the National Institutes of Health, National Institute of General Medical Sciences (GM40998 and GM52811). We would like to thank Dr. Fu-Tyan Lin (University of Pittsburgh) for the ^{13}C nmr spectra.

REFERENCES AND NOTES

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[1] Taken in part from the thesis submitted by L. C. to the Graduate School of Pharmaceutical Sciences, Duquesne University, in partial fulfillment of the requirements for the degree of Master of Science, June 1995.

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