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Syntheses of Polyamides Containing Theophylline and Thymine

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SUMMARY:

2-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)methylsuccinic acid (**2a**) and 2-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methylsuccinic acid (**2b**) were synthesized via the addition reaction of theophylline and thymine, respectively, to dimethyl methylenesuccinate, followed by hydrolysis of the resulting ester. The dicarboxylic acid derivatives **2a** and **2b** were further converted to their di-*p*-nitrophenyl esters **3a** and **3b**, which were allowed to polycondense with diamines such as 1,6-diaminohexane, 1,2-diaminoethane, 3-aminomethylbenzylamine, and piperazine in solutions. The resulting polyamides are white powders with molecular weights in the range of about 2000–6000. The $\overline{DP}$ of the polyamides varies with the kind of diamines and solvent used. All polyamides are soluble in DMSO and formic acid, the polyamides deriving from esters **3a** and **3b** and 1,2-diaminoethane and piperazine are also soluble in water.

Introduction

Various kinds of model compounds of polynucleotides have been synthesized by several manners and have provided us much about nucleic acids chemistry.

The synthesis of some polynucleotide analogs of which the main chains consist of phosphoric diester, triester, and ester linkages by polycondensation of bifunctional compounds such as diol, phosphoric acid, and hydroxycarboxylic acid derivatives of nucleic acid bases, have previously been reported[1–4]. These synthetic analogs are simple models of nucleic acids and the interaction with natural nucleic acids has been observed in aqueous solutions. Recently, the polyamides having theophylline and uracil have been synthesized through polycondensation of their diethyl malonate derivatives with diamines. This analog was found to have a molecular weight of 1000–1200[5].

As further study on the synthesis of polynucleotide analogs, we wish to describe the syntheses of some polyamides, having theophylline and thymine as side groups, via polycondensations of di-*p*-nitrophenyl succinate derivatives of them with various diamines. These polyamides containing nucleic acid bases will be expected to be model compounds of polynucleotides with both high molecular weights and water solubilities.

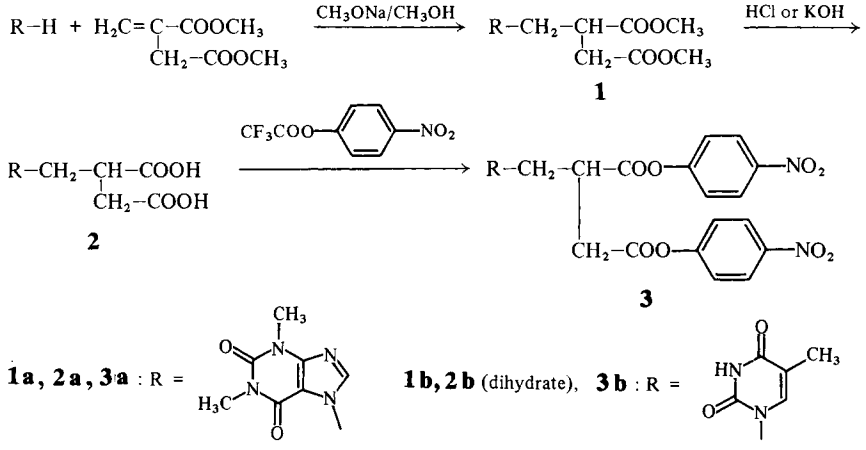
Results and Discussion

Syntheses of dicarboxylic acids **2a** *and* **2b** *and their di-p-nitrophenyl esters* **3a** *and* **3b** *of theophylline and thymine*

Theophylline and thymine were first converted, respectively, to the dimethyl ester derivatives **1a** and **1b**, followed by hydrolysis to the corresponding dicarboxylic acid derivatives **2a** and

2b, which were further converted to their di-*p*-nitrophenyl esters **3a** and **3b** with *p*-nitrophenyl trifluoroacetate. The preparative route is shown in Scheme 1.

Scheme 1:

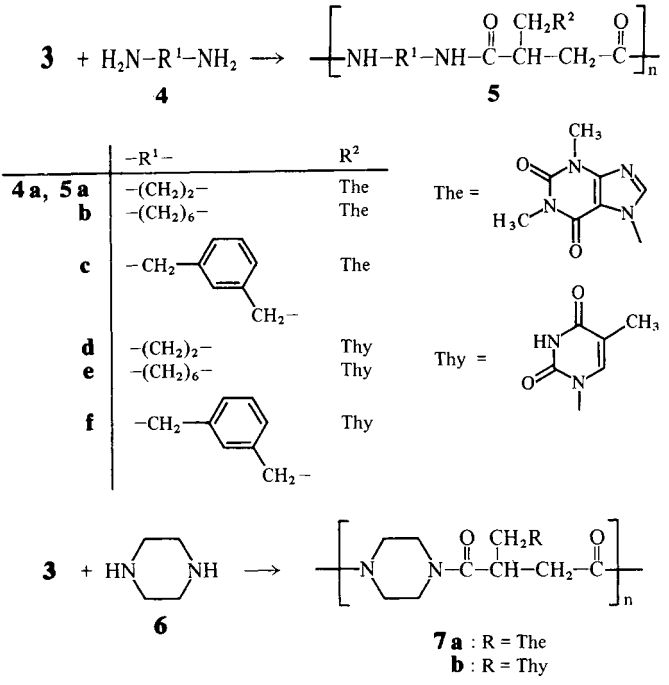


Lira et al.[6] have reported that adenine was allowed to react with some electrophilic olefins such as ethyl acrylate and acrylonitrile under base-catalyzed conditions to afford the corresponding 9-alkylated adenine derivatives in high yield. In addition, the cyanoethylation of uracil and a few nucleosides with acrylonitrile has been reported[7]. Theophylline was added to dimethyl methylenesuccinate in refluxing methanol containing a trace of sodium methoxide to afford the 7-alkylated theophylline derivative **1a** in 83% yield. The dimethyl ester **1a** was easily hydrolyzed with diluted hydrochloric acid to the dicarboxylic acid **2a** in 80% yield. Similarly, the reaction of thymine with dimethyl methylenesuccinate under the same conditions gave mainly the 1-alkylated thymine derivative **1b** in 64% yield and a trace of the 3-isomer which was not isolated but characterized by thin layer chromatography and by its UV spectrum. The dimethyl ester **1b** was also hydrolyzed with potassium hydroxide in methanol to the dicarboxylic acid **2b** in 78% yield. The dicarboxylic acids **2a** and **2b** were successfully converted to the corresponding di-*p*-nitrophenyl esters **3a** and **3b**, respectively, by ester-exchange reaction with *p*-nitrophenyl trifluoroacetate in anhydrous pyridine, according to the literature on the preparation of *p*-nitrophenyl esters of ordinary carboxylic acids and acylated amino acids[8,9]. The compounds were characterized by their IR, UV, and NMR spectra and elemental analyses, as shown in the Exptl. Part.

Polycondensations of esters **3a** *and* **3b** *with diamines*

The active ester method, widely used in peptide[10] and polyamide syntheses[11], was applied to the syntheses of polyamides containing theophylline and thymine residues. The polycondensations of esters **3a** and **3b** with diamines such as 1,2-diaminoethane, 1,6-diaminohexane, 3-aminomethylbenzylamine, and piperazine were carried out in various solvents at 30°C for 24 or 48 h (Scheme 2).

Scheme 2:



All obtained polyamides were purified by reprecipitation from formic acid/acetone. The polyamides **5** and **7** were characterized by IR spectroscopy and elemental analyses. The IR spectra of ester **3a** and polyamide **5b**, obtained by polycondensation of **3a** with 1,6-diaminohexane (**4b**) in DMF are given in Fig. 1. The spectrum of ester **3a** shows the absorption peaks at 1520, 1350, and 860 cm^{-1}, due to the nitro groups, which has disappeared in the spectrum of **5b**, in which new peaks at 3250 cm^{-1} due to the NH of amide groups and at 2800 cm^{-1} due to the hexamethylene groups appeared. The results of polycondensations of esters **3a** and **3b** with various diamines are summarized in Tab. 1. The molecular weights of the polyamides **5** and **7** were determined by vapor pressure osmometry and found to have a range of about 2000–6000. Expectedly, the prepared polyamides have higher molecular weights than the polynucleotide analogs consisting of polyphosphoric diester and polyester backbone, reported before. The polycondensation reactions proceeded homogeneously in aprotic polar solvents such as DMSO, DMF, or *N*-methyl-2-pyrrolidone, but heterogeneously in ethanol and 1,4-dioxane. Consequently, the $\overline{DP}$s and yields of the polyamides prepared in aprotic polar solvents were higher than those prepared in ethanol and 1,4-dioxane. Polycondensation in DMSO yielded polyamides with a variety of $\overline{DP}$s depending on the diamine and increasing in the following order: **4b** > **4c** ≈ **4a** > **6**. These results show the appearance of steric hindrance of the nucleic acid base moieties in this polycondensation reaction. The polyamides were obtained as white and sparingly hygroscopic powders, soluble in DMSO and formic acid, and insoluble in common organic solvents such as ethanol and acetone. Interestingly, polyamides **5a, d,** and **7a, b,** derived from **4a** and **6** were also soluble in water and showed UV absorptions at 273 and

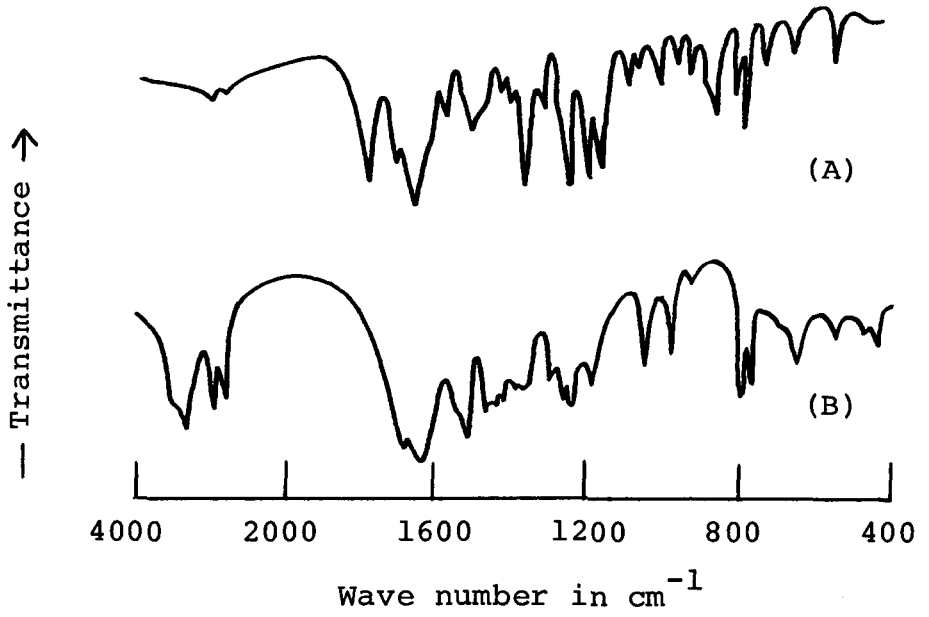


Fig. 1. IR spectra of (A): di-*p*-nitrophenyl 2-(theophyllin-7-yl)methylsuccinate (**3a**); (B): polyamide **5b** (poly[iminohexamethyleneimino-2-(theophyllin-7-yl)methylsuccinyl])

Tab. 1. Results of the polycondensation of ester **3a** and **3b** with various diamines at 30 °C for 24 h

Ester	Diamine[a]	Solvent[b]	Polyamide	Yield in %	*M*	$\overline{DP}$
3a	DAH	Ethanol	**5b**	52	1600	4,1
	DAH	1,4-Dioxane		44	1300	3,3
	DAH	DMF		89	5400	13,8
	DAH	NMP		89	5200	13,3
	DAH	DMSO		95	6700	17,2
	DAE	NMP	**5a**	50	2800	8,4
	DAE	DMSO		56	3000	9,0
	ABA	DMSO	**5c**	82	4400	10,7
	Piperazine	DMSO	**7a**	72[c]	2100	5,8
3b	DAH	NMP	**5e**	79	4800	14,3
	DAH	DMSO		85	5200	15,5
	DAE	NMP	**5d**	58	3100	11,1
	DAE	DMSO		62	3200	11,4
	ABA	DMSO	**5f**	90	4200	11,8
	Piperazine	DMF	**7b**	86[c]	2300	7,5
	Piperazine	DMSO		82[c]	1600	5,2

[a] DAH: 1,6-diaminohexane; DAE: 1,2-diaminoethane; ABA: 3-aminomethylbenzylamine.
[b] NMP: *N*-methyl-2-pyrrolidone.
[c] Reaction time: 48 h.

269 nm, due to the theophylline and thymine rings as side groups, respectively. The polyamides **5** and **7** obtained from the polycondensation in DMSO, melted at about 200 and 250 °C, respectively. Their λ_{max} values of the UV spectra, melting points, and elemental analyses are listed in Tab. 2.

Tab. 2. Elemental analyses and some physical properties of the polyamides **5** and **7**

Poly-amide	$\lambda_{max}^{H_2O}$ in nm	M. p. in °C	Formula		Analyses		
5b	—	169–174	$(C_{18}H_{26}N_6O_4)_n$	Calc.[a]	C 52,59	H 6,68	N 19,90
				Found	C 52,38	H 6,36	N 20,33
5a	273	198–208	$(C_{14}H_{18}N_6O_4)_n$	Calc.[b]	C 46,39	H 5,64	N 22,39
				Found	C 46,12	H 5,26	N 22,90
5c	—	194–208	$(C_{20}H_{22}N_6O_4)_n$	Calc.[a]	C 55,65	H 5,47	N 19,00
				Found	C 55,83	H 5,51	N 19,12
7a	273	233–244	$(C_{16}H_{20}N_6O_4)_n$	Calc.[b]	C 49,37	H 5,78	N 20,94
				Found	C 49,08	H 5,21	N 21,25
5e	—	188–200	$(C_{16}H_{24}N_4O_4)_n$	Calc.[a]	C 53,79	H 7,11	N 15,21
				Found	C 53,67	H 6,91	N 15,75
5d	269	189–198	$(C_{12}H_{16}N_4O_4)_n$	Calc.[b]	C 46,72	H 5,96	N 17,44
				Found	C 46,32	H 5,54	N 17,79
5f	—	198–217	$(C_{18}H_{20}N_4O_4)_n$	Calc.[b]	C 55,91	H 5,83	N 14,10
				Found	C 56,08	H 5,47	N 14,60
7b	269	255–265	$(C_{14}H_{18}N_4O_4)_n$	Calc.[b]	C 50,14	H 5,09	N 16,13
				Found	C 49,92	H 5,85	N 16,02

[a] Calc. for the formula $+1/2\ (HCOOH + H_2O)$.
[b] Calc. for the formula $+1/2\ (HCOOH) + H_2O$.

Experimental Part

Melting points are uncorrected. The IR spectra were run on a JASCO Model IR-G spectrometer. The UV spectra were measured by a Hitachi Model EPS-3T spectrometer. The NMR spectra were recorded with Hitachi-Perkin-Elmer Model R-20.

Materials: Commercial theophylline, thymine, and dimethyl methylenesuccinate were used without further purification. All solvents were purified as usual. *p*-Nitrophenyl trifluoroacetate was prepared from *p*-nitrophenol and trifluoroacetic anhydride according to the procedure of Sakakibara et al.[8].

Preparation of monomers

Dimethyl 2-(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)methylsuccinate (**1a**): A mixture of theophylline (7,20 g, 40 mmol) and a trace of sodium hydride (50 weight-% in mineral oil) in abs. methanol (70 ml) was stirred at room temperature for 1 h. Then, dimethyl methylenesuccinate (3,16 g, 20 mmol) was added to the suspension and the mixture was refluxed for 40 h. After allowing the reaction mixture to cool in a refrigerator overnight, excess theophylline precipitated was filtered off. The filtrate was evaporated i. vac. to dryness, and the residual semi-solid was dissolved in chloroform (200 ml). The solution was washed with water until contaminated theophylline was completely removed from the mixture. The chloroform solution was evaporated i. vac. and the residue was solidified with benzene/hexane. The solid was recrystallized from benzene/hexane. Colorless needles; mp 90–91 °C. Yield: 5,60 g (83%).

UV (methanol): λ_{max} 273 nm ($\varepsilon = 7200\ l \cdot mol^{-1} \cdot cm^{-1}$).

IR (KBr): 1740 cm^{-1} (s; C=O).

^{1}H NMR ($CDCl_3$): τ=2,30 (s; C^8-H), 5,30 (d; >N—CH_2—), 6,25 (s; —$COOCH_3$), 6,40 and 6,60 (s; N—CH_3), 6,50 (m; —CH<), and 7,25 (d; —CH_2—COO—).

$C_{14}H_{18}N_4O_6$ (338,3)	Calc.	C 49,70	H 5,36	N 16,56
	Found	C 49,44	H 5,36	N 16,66

2-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)methylsuccinic acid (**2a**): Compound **1a** (3,38 g, 10 mmol) in 2 M hydrochloric acid (50 ml) was refluxed for 4 h and then evaporated i. vac. to dryness. An aqueous solution of sodium carbonate was added to the residue and the solution was adjusted to pH 3 with dil. hydrochloric acid. The precipitated solid was filtered off, washed with ice/water and recrystallized from methanol. Colorless needles; mp 206–207 °C. Yield: 2,73 g (88%).

UV (water): λ_{max} 273 nm (ε = 8600 l · mol^{-1} · cm^{-1}).

^{1}H NMR (D_2O): τ=2,00 (s; C^8-H), 5,40 (d; >N—CH_2—), 6,55 and 6,75 (s; N—CH_3), 6,60 (m; —CH<), and 7,30 (d; —CH_2—COO—).

$C_{12}H_{14}N_4O_6$ (310,3)	Calc.	C 46,45	H 4,55	N 18,06
	Found	C 46,30	H 4,69	N 17,78

Di-p-nitrophenyl 2-(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)methylsuccinate (**3a**): A mixture of compound **2a** (1,55 g, 5 mmol) and *p*-nitrophenyl trifluoroacetate (2,80 g, 12 mmol) in anhydrous pyridine (5 ml) was stirred at 30 °C for 48 h. Water (40 ml) was added to the reaction mixture and kept in a refrigerator for several hours. The resulting precipitate was filtered off and washed with methanol. The solid was recrystallized from ethyl acetate. Colorless needles; mp 190 °C. Yield: 2,22 g (80%).

UV (methanol): λ_{max} 274 nm (ε = 23000 l · mol^{-1} · cm^{-1}).

IR (KBr): 1765 (s; C=O), 1520, 1350, and 860 cm^{-1} (s; NO_2).

^{1}H NMR (DMSO-d_6): τ=1,85 (s; C^8-H), 1,75–2,65 (m; *p*-nitrophenyl proton), 5,20 (d; >N—CH_2—), 6,10 (m; >CH—), 6,55 and 6,75 (N—CH_3), and 6,80 (d; —CH_2—COO—).

$C_{24}H_{20}N_6O_{10}$ (552,5)	Calc.	C 52,17	H 3,65	N 15,21
	Found	C 52,04	H 3,77	N 14,92

Dimethyl 2-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methylsuccinate (**1b**): The same reaction conditions and procedures as those for the compound **1a** were applied. Recrystallization from benzene gave colorless needles; mp 143–144 °C. Yield: 64%.

UV (methanol): λ_{max} 269 nm (ε = 9400 l · mol^{-1} · cm^{-1}).

IR (KBr): 1725 cm^{-1} (vs; C=O).

^{1}H NMR ($CDCl_3$): τ = −0,10 (s; N—H), 2,90 (s; C^6-H), 6,00 (d; >N—CH_2—), 6,30 (s; —$COOCH_3$), 6,50 (m; >CH—), 7,30 (d; —CH_2—COO—), and 8,10 (s; C^5-CH_3).

$C_{12}H_{16}N_2O_6$ (284,3)	Calc.	C 50,70	H 5,67	N 9,86
	Found	C 50,50	H 5,42	N 9,78

2-(5-Methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methylsuccinic acid dihydrate (**2b**): A mixture of compound **1b** (4,26 g, 15 mmol) and potassium hydroxide (3,30 g, 59 mmol) in abs. methanol (20 ml) was stirred at 30 °C for 70 h. The mixture was evaporated and the residue was dissolved in a small volume of water. The solution was adjusted to pH 3 with dil. hydrochloric acid and kept in a refrigerator overnight. The resulting precipitate was filtered off and recrystallized from water. Colorless needles; mp 245–247 °C. Yield: 3,40 g (78%).

UV (methanol): λ_{max} 269 nm (ε = 9400 l · mol^{-1} · cm^{-1}).

^{1}H NMR (DMSO-d_6): τ = −1,15 (s; N—H), 0,50 (—COOH and H_2O), 2,55 (s; C^6-H), 6,20 (d; >N—CH_2—), 6,95 (m; >CH—), 7,50 (d; —CH_2—COO—), and 8,25 (s; C^5-CH_3).

$C_{10}H_{12}N_2O_6 \cdot 2H_2O$ (292,2)	Calc.	C 41,10	H 5,52	N 9,59
	Found	C 41,22	H 5,82	N 9,35

Di-p-nitrophenyl 2-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)methylsuccinate (**3b**): The same reaction conditions and procedures as those for compound **3a** were applied. Colorless needles; mp 184–185 °C. Yield: 70%.

UV (methanol): λ_{max} 270 nm (ε = 26000 l · mol^{-1} · cm^{-1}).

IR (KBr): 1760 (s; C=O), 1525, 1350, and 860 cm^{-1} (vs; NO_2).

^{1}H NMR (DMSO-d_6); $\tau = -1{,}14$ (s; N—H), 1,65 and 2,60 (d; *p*-nitrophenyl proton), 2,35 (s; C^6-H), 5,85 (d; $>N-CH_2-$), 6,85 (m; $>CH-$), 7,50 (d; $-CH_2-COO-$), and 8,25 (s; C^5-CH_3).

$C_{22}H_{18}N_4O_{10}$ (498,4)	Calc.	C 53,01	H 3,64	N 11,24
	Found	C 52,92	H 3,61	N 11,08

General polycondensation procedure: A mixture of di-*p*-nitrophenyl ester **3a** or **3b** and diamine (mole ratio: 1:1) was stirred at 30°C in several solvents. After a given period of polycondensation, the reaction mixture was poured into a large excess of acetone/water (volume ratio: 9/1) and further stirred at 30°C overnight. The precipitated polyamide was collected and washed with acetone. All polyamides obtained were purified by reprecipitation from formic acid/acetone.

Vapor pressure osmometry: The molecular weights of polyamides were measured with a vapor pressure osmometer from Knauer using formic acid as a solvent at 55°C.

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