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# Synthesis and Polymerization of Methacryl Esters Having Two Kinds of Nucleic Acid Bases

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SUMMARY:

Methacrylic acid esters having two different and same kinds of nucleic acid bases such as theophylline-theophylline, theophylline-thymine, theophylline-uracil, and uracil-uracil, were synthesized, and polymerized in DMF or DMSO at 70 °C using 2,2'-azoisobutyronitrile as an initiator. The polymers obtained were found to have molecular weights in the range of about 1 800–4 000, according to vapor pressure osmometry and gel filtration measurement. The white powdery polymers were soluble in DMSO, DMF, and trifluoroacetic acid, but insoluble in water, alcohols, and common organic solvents.

## *Introduction*

On the purpose of making clear the behavior and function of nucleic acids, the synthesis and polymerization of various kinds of vinyl type monomers containing nucleic acid bases have been accomplished by many workers. On the other hand, Seita et al.[1,2] have prepared some dinucleotide analogs **2** in which the bases are connected by a trimethylene chain, in order to study the interactions of nucleic acid bases in the absence of complicating factors associated with hydrogen bonding or the usual carbohydrate and phosphoric diester linkages.

As an extention of the investigation on polynucleotide analogs, the author made an attempt to prepare polymers containing these dinucleotide analogs, which were obtained by the polymerization of the corresponding methacrylic acid esters **3** of dinucleotide analogs **2**. These polymers are new polynucleotide analogs with pending two different or same kinds of nucleic acid bases in the ratio of 1/1.

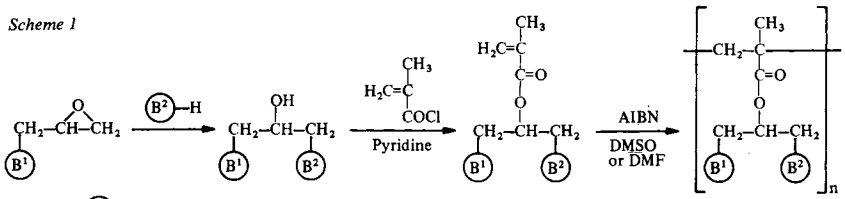
## *Results and Discussion*

### 1. *Preparation of methacrylic acid esters* **3a–d**

Dinucleotide analogs **2a–c** containing the theophylline ring as a component were conveniently prepared by the modified procedure of Seita et al.[2]. Thus, dinucleotide analogs **2a–c** such as theophylline-theophylline, theophylline-thymine, and theophylline-uracil were prepared by the addition reaction of theophylline, thymine, and uracil to 1-(theophyllin-7-yl)-2,3-epoxypropane (**1**) which was obtained by the reaction of the sodium salt of theophylline with an excess of 1-chloro-2,3-epoxypropane.

The reaction of the compound **1** with theophylline was carried out in DMF containing a trace amount of anhydrous potassium carbonate to afford the dinucleotide analog **2a** which was substituted at 7-N position of the theophylline ring, in excellent yield. In the cases of pyrimidine bases such as thymine and uracil, the mixture of dinucleotide analogs and the unde-

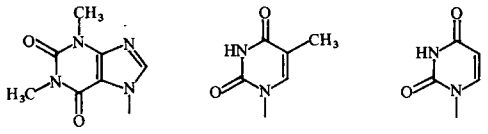
*Scheme 1*




1 ; (B¹) = The 2 3 4

| 2-4 | (B¹) | (B²) |
|---|---|---|
| **a** | The | The |
| **b** | The | Thy |
| **c** | The | Ura |
| **d** | Ura | Ura |

(B) =



(The) (Thy) (Ura)

sired product in which the 3-N position of pyrimidine rings probably participated, respectively, was produced under the same reaction condition as that for theophylline. The desired dinucleotide analogs **2b** and **2c** were easily purified by the fractional recrystallization of the mixture. Takemoto et al.[3] have also reported that the reaction of adenine with epoxides such as propylene oxide and styrene oxide in DMF containing sodium hydroxide gave the 9-substituted adenine derivatives through the ring opening at a less hindered site of the epoxide. Dinucleotide analog **2d** containing uracil-uracil was prepared according to the literature[1]. The structures of the dinucleotide analogs **2a–d** were identified by comparing their IR and UV spectra with those of the authentic samples, obtained by the procedures of Seita et al., and were further confirmed by their $^1$H NMR spectra and elemental analyses.

The dinucleotide analogs **2a–d** were allowed to react with methacryloyl chloride in anhydrous pyridine under several conditions, giving the corresponding methacrylic acid ester derivatives **3a–d**. The reaction of equimolar amounts of compounds **2a–d** and methacryloyl chloride in anhydrous pyridine at 0 °C gave the desired products in less than 10% yield and most of the starting materials were recovered. Also on heating at 80 °C, there were considerable amounts of unknown products which were probably acylated at heterocyclic moieties, so that the subsequent isolation of the desired product was difficult. The best results were obtained when the reaction was conducted at room temperature using an excess of methacryloyl chloride in anhydrous pyridine. The methacrylic acid esters **3a–d** were purified by the combination of column chromatography and recrystallization. The structures of compounds **3a–d** were characterized by the IR-, UV-, and $^1$H NMR spectra and elemental analyses, as shown in the Exptl. Part. The IR spectra indicated the existence of methacrylate and nucleic acid base groups. The UV spectra showed almost the same $\lambda_{max}$ values as those of the corresponding dinucleotide analogs. The chemical shifts observed in the $^1$H NMR spectra were corresponding to that for protons of the usual compounds with methacrylate, purine, and pyrimidine groups.

## 2. *Polymerization of methacrylic acid esters* **3a–d**

The polymerization of methacrylic acid esters **3a–d** was carried out in DMSO and DMF at 70 °C for a given time, using 2,2′-azoisobutyronitrile (AIBN) as an initiator. The results of polymerization are summarized in Tab. 1.

Tab. 1. Results of the polymerization of methacrylic acid esters **3a–d** at 70 °C [a]

| Monomer | Solvent | Time in h | Conversion in % | $[\eta]$ [b] in dl/g | $M_n$ | $\overline{DP}$ |
|---|---|---|---|---|---|---|
| **3a** $B^1$ = The, $B^2$ = The | DMF | 20 | 25 | 0,07 | 3800 [c] | 7,8 |
| | DMSO | 5 | 18 | — | — | — |
| | DMSO | 20 | 66 | 0,07 | — | — |
| **3b** $B^1$ = The, $B^2$ = Thy | DMSO | 10 | 25 | — | — | — |
| | DMSO | 20 | 32 | 0,08 | 4000 [c] | 9,3 |
| **3c** $B^1$ = The, $B^2$ = Ura | DMF | 20 | 21 | — | — | — |
| | DMSO | 20 | 38 | 0,06 | 2400 [d] | 5,7 |
| | DMSO | 10 | 15 | — | — | — |
| **3d** $B^1$ = Ura, $B^2$ = Ura | DMF | 20 | 12 | — | — | — |
| | DMSO | 20 | 29 | 0,07 | 1800 [d] | 5,1 |

[a] Monomer: 200 mg; AIBN: 5 mg; Solvent: 2 ml.
[b] Measured in DMF at 30 °C.
[c] Measured by vapor pressure osmometry in DMF at 90 °C.
[d] Measured by vapor pressure osmometry in formic acid at 55 °C.

All polymers were purified by reprecipitation from DMSO/methanol and obtained as white powders. They are soluble in DMSO, DMF, and trifluoroacetic acid and insoluble in water, alcohols, and common hydrocarbons. Besides the $[\eta]$ values of polymers, the molecular weights of polymers, obtained by the polymerization in DMSO, were measured by gel filtration and va-

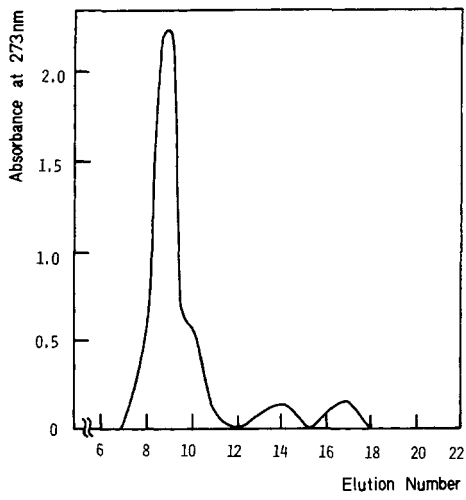



Fig. 1. Gel filtration pattern of polymer **4a**. Column: Sephadex LH-20; Solvent: DMF; Fraction volume: 2,0 ml; Elution rate: 8 ml/10 min; Column bed.: 1,5 · 60 cm; Internal standard: Trypan Red (MW 1003) in elution number 9 and 10

por pressure osmometry. By gel filtration measurement on Sephadex LH-20, using Trypan Red (molecular weight 1003) as an internal standard, it has been found that most of these polymers have molecular weights of more than 1000. As an example, the gel filtration pattern of polymer **4a** is given in Fig. 1.

In this figure, most of the polymer **4a** was eluted in elution numbers 7–12 and Trypan Red was eluted in elution numbers 9 and 10. The peaks at elution numbers 13–17 represent polymer fractions with lower molecular weights, because the absence of residual monomer in the resulting polymer was confirmed by thin layer chromatography. Also, the molecular weights of the polymers, determined by vapor pressure osmometry, were given in Tab. 1.

## *Experimental Part*

Melting points were determined in an open capillary and 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 a Hitachi-Perkin-Elmer Model R-20.

### *Materials*

Commercially available theophylline, thymine, uracil, and 1-chloro-2,3-epoxypropane were used without further purification. All solvents were purified as usual. Methacryloyl chloride was prepared from methacrylic acid and benzoyl chloride[4].

### *Preparation of 2-propanol derivatives*

*1,3-Bis(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-2-propanol* (**2a**): A mixture of 1-(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-2,3-epoxypropane (**1**)[5] (4,72 g; 20 mmol), theophylline (3,6 g; 20 mmol), and a trace amount of anhydrous potassium carbonate in DMF (100 ml) was stirred at 80 °C for 12 h. The reaction mixture was evaporated i. vac. to dryness and the residual solid was recrystallized from methanol. Colorless needles; mp 283–285 °C (Lit.[5] 283–285 °C). Yield: 6,74 g (82%).

UV ($H_2O$): $\lambda_{max}$ 274 nm ($\varepsilon = 16\,800\ l \cdot mol^{-1} \cdot cm^{-1}$).

| | | | | |
|---|---|---|---|---|
| $C_{17}H_{20}N_8O_5$ (416,2) | Calc. | C 49,06 | H 4,84 | N 26,93 |
| | Found | C 48,99 | H 4,84 | N 26,85 |

*1-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-propanol* (**2b**): A mixture of compound **1** (3,54 g; 15 mmol), thymine (1,9 g; 15 mmol), and a trace amount of potassium carbonate in DMF (40 ml) was stirred at 100 °C for 7 h. The reaction mixture was evaporated i. vac. to dryness and ethanol (10 ml) was added to the oily residue. The precipitated solid was filtered off and recrystallized from ethanol. Colorless needles; mp 256–257 °C. Yield: 1,63 g (31%).

UV ($H_2O$): $\lambda_{max}$ 274 nm ($\varepsilon = 15\,600\ l \cdot mol^{-1} \cdot cm^{-1}$).

$^1$H NMR (DMSO-$d_6$): $\tau$ = 2,00 (s, $C^8-H$ of theophylline ring), 2,60 (s, $C^6-H$ of thymine ring), 4,54 (s, —OH), 5,54–6,48 (m, $—CH_2—\underset{|}{CH}—CH_2—$), 6,58 and 6,78 (s, N—$CH_3$), and 8,26 ppm (s, $C^5-CH_3$ of thymine ring).

| | | | | |
|---|---|---|---|---|
| $C_{15}H_{18}N_6O_5$ (362,3) | Calc. | C 49,72 | H 5,02 | N 23,20 |
| | Found | C 49,47 | H 5,28 | N 22,94 |

*1-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-propanol* (**2c**): A mixture of compound **1** (7,08 g; 30 mmol), uracil (3,36 g; 30 mmol), and a trace amount of anhydrous potassium carbonate in DMF (100 ml) was stirred at 100 °C for 8 h. The reaction mixture was kept in a refrigerator overnight and the resulting precipitate was filtered off. Recrystallization from water gave colorless needles; mp 300 °C. Yield: 2,02 g (20%).

UV ($H_2O$): $\lambda_{max}$ 270 nm ($\varepsilon = 13\,500\ l \cdot mol^{-1} \cdot cm^{-1}$).

$^1$H NMR (DMSO-$d_6$); $\tau$ = 2,04 (s, $C^8-H$ of theophylline ring), 2,51 (d, $C^5-H$ of uracil ring), 4,50 (d, $C^6-H$ of uracil ring), 4,51 (s, —OH), 5,78–6,46 (m, $—CH_2—\underset{|}{CH}—CH_2—$), and 6,60 and 6,80 ppm (s, N—$CH_3$).

| | | | | |
|---|---|---|---|---|
| $C_{14}H_{16}N_6O_5$ (348,3) | Calc. | C 48,27 | H 4,64 | N 24,14 |
| | Found | C 48,27 | H 4,89 | N 24,54 |

*1,3-Bis(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-propanol* (**2d**): Compound **2d** was prepared according to the procedure of Seita et al.[1]. Colorless needles; mp 140–142 °C. (Lit.[1] mp 139–142 °C).

UV ($H_2O$): $\lambda_{max}$ 266,5 nm ($\varepsilon = 19300\ l \cdot mol^{-1} \cdot cm^{-1}$).

| | | | | |
|---|---|---|---|---|
| $C_{11}H_{12}N_4O_5$ (280,2) | Calc. | C 47,15 | H 4,32 | N 20,00 |
| | Found | C 46,97 | H 4,52 | N 19,65 |

*Preparation of monomers*

*Preparation procedures of methacrylic acid esters* **3a–d**: Freshly distilled methacryloyl chloride (1,2 ml) was added dropwise to the suspension of 2-propanol derivatives **2a–d** (10 mmol) in anhydrous pyridine (250 ml) with stirring at 0 °C. The mixture was stirred at room temperature for 24 h and then additional methacryloyl chloride (0,5 ml) was added to the mixture, which was further stirred for 10 h at room temperature. The reaction mixture was filtered and the filtrate was evaporated i. vac. to dryness. Ethanol (10 ml) was added to the residue and the solution was kept in a refrigerator overnight. The resulting precipitate was filtered off and the solid was chromatographed on silica gel. Elution with benzene/ethanol (volume ratio: 4/1) gave the product, which was recrystallized from suitable solvents.

*1,3-Bis(1,3-dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-2-propyl methacrylate* (**3a**): Recrystallization from methanol/water gave colorless needles; mp 123–125 °C. Yield: 33%.

UV ($H_2O$): $\lambda_{max}$ 273 nm ($\varepsilon = 16900\ l \cdot mol^{-1} \cdot cm^{-1}$).

IR (KBr): 1720 (s; C=O) and 1145 $cm^{-1}$ (s; C—O).

$^1H$ NMR (DMSO-$d_6$): $\tau = 1{,}82$ (s, $C^8-H$), 4,00–4,33 (m, $H_2C{=}C<$ and $>CH—$), 5,40–5,50 (m, $>N—CH_2—$), 6,53 and 6,73 (s, $N—CH_3$), and 8,20 (s, $>C{=}C<^{CH_3}$).

| | | | | |
|---|---|---|---|---|
| $C_{21}H_{24}N_8O_6 \cdot 2H_2O$ (502,5) | Calc. | C 50,19 | H 5,22 | N 22,30 |
| | Found | C 50,05 | H 4,90 | N 22,34 |

*1-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-(5-methyl-2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-propyl methacrylate* (**3b**): Recrystallization from ethanol/water gave colorless needles; mp 178–179 °C. Yield: 25%.

UV ($H_2O$): $\lambda_{max}$ 274 nm ($\varepsilon = 15600\ l \cdot mol^{-1} \cdot cm^{-1}$).

IR (KBr): 1710 (s, C=O) and 1155 $cm^{-1}$ (s, C—O).

$^1H$ NMR (DMSO-$d_6$): $\tau = 1{,}81$ (s, $C^8-H$ of theophylline ring), 2,54 (s, $C^6-H$ of thymine ring), 3,96–4,25 (m, $H_2C{=}C<$ and $>CH—$), 5,35–5,69 (m, $>N—CH_2—$), 6,56 and 6,75 (s, $>N—CH_3$), 8,25 and 8,30 ppm (s, $C^5-H$ of thymine ring and $>C{=}C<^{CH_3}$).

| | | | | |
|---|---|---|---|---|
| $C_{19}H_{22}N_6O_6 \cdot 3/2\,H_2O$ (457,4) | Calc. | C 49,89 | H 5,51 | N 18,38 |
| | Found | C 50,24 | H 5,54 | N 17,83 |

*1-(1,3-Dimethyl-2,6-dioxo-2,6-dihydropurin-7-yl)-3-(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-propyl methacrylate* (**3c**): Recrystallization from ethanol/water gave colorless needles; mp 243–245 °C. Yield: 25%.

UV ($H_2O$): $\lambda_{max}$ 270 nm ($\varepsilon = 13600\ l \cdot mol^{-1} \cdot cm^{-1}$).

IR (KBr): 1708 (s; C=O) and 1150 $cm^{-1}$ (s; C—O).

$^1H$ NMR (DMSO-$d_6$): $\tau = 1{,}82$ (s, $C^8-H$ of theophylline ring), 2,40 (d, $C^5-H$ of uracil ring), 3,94–4,44 (m, $C^6-H$ of uracil ring, $H_2C{=}C<$, and $>CH—$), 5,30–5,85 (m, $>N—CH_2—$), 6,55 and 6,75 (s, $>N—CH_3$), and 8,22 ppm (s, $>C{=}C<^{CH_3}$).

| | | | | |
|---|---|---|---|---|
| $C_{18}H_{20}N_6O_6 \cdot 3/2\,H_2O$ (443,4) | Calc. | C 48,80 | H 5,23 | N 18,96 |
| | Found | C 48,63 | H 5,09 | N 18,54 |

*1,3-Bis(2,4-dioxo-1,2,3,4-tetrahydropyrimidin-1-yl)-2-propyl methacrylate* (**3d**): Recrystallization from water gave colorless needles; mp 250–252 °C. Yield: 20%.

UV ($H_2O$): $\lambda_{max}$ 266 nm ($\varepsilon = 18900\ l \cdot mol^{-1} \cdot cm^{-1}$).

IR (KBr): 1706 (s; C=O), and 1150 $cm^{-1}$ (s, C—O).

$^1$H NMR (DMSO-$d_6$): $\tau$ = 2,41 (d, $C^5-H$), 3,95–4,45 (m, $C^6-H$, $H_2C{=}C<$, and $>CH-$), 5,32–5,78 (m, $>N-CH_2-$), and 8,26 ppm (s, $>C{=}C<^{CH_3}$).

| | | | | |
|---|---|---|---|---|
| $C_{15}H_{16}N_4O_6 \cdot H_2O$ (366,3) | Calc. | C 49,18 | H 4,95 | N 15,30 |
| | Found | C 49,31 | H 5,01 | N 15,22 |

*Polymerization of monomers* **3a–d**

Polymerization was carried out in a sealed glass tube after flushing with nitrogen and evacuating the contents. After the mixture of monomer (200 mg) and 2,2′-azoisobutyronitrile (5 mg) in DMF or DMSO (2 ml) was shaken at 70 °C for a given period, the contents were added into excess methanol to precipitate the polymer, which was purified by reprecipitation from DMSO/methanol.

*Vapor pressure osmometry*

The molecular weights of polymers were measured with a vapor pressure osmometer fom Knauer using formic acid as a solvent at 55 °C.

*Gel filtration measurement*

The molecular weights of polymers were also determined by means of gel filtration on a Sephadex LH-20 column using DMF as an eluate. The contents of polymers in each fraction were followed by UV absorption at suitable wavelength. Trypan Red (molecular weight 1003) was used as an internal standard for the gel filtration. The count of elution numbers was begun after about 30 ml of solvent had been eluted.

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