Supplementary Material for Organic & Biomolecular Chemistry


# Supplementary data

**Synthetic procedure for the 5'-triphosphates of thymidine analogues, 4-14 and 16.**

**5-N-(6-[4-Imidazoleacetyl]aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (4).** To a suspension of sodium imidazole 4-acetate (148 mg, 1.0 μmol) in dry THF (5.0 ml), a solution of 2,4-dinitrofluorobenzene (465 mg, 2.5 mmol) in dry THF (10 ml) was added gradually with stirring. The solution was stirred overnight at room temperature. The reaction mixture was poured into hexane and the resulting precipitate was collected with filtration. The precipitate was dissolved in THF and poured again into hexane, and the precipitate was collected with a glass filter, washed with hexane and dried in a desiccator to give 1-(2,4-dinitrophenyl)-imidazole 4-acetic acid as a yellow powder. The yield was 186 mg (64 %). 1H NMR (CD3OD) δ 8.94 (1H, d, J=2.7 Hz, DNP-H3), 8.64(1H, dd, DNP-H5), 7.93(1H, d, J=9.0 Hz, DNP-H6), 7.91(1H, s, Im-H2), 7.31(1H, s, Im-H5), 3.65(2H, s, CH2). ESI-Mass (positive mode) *e/z*: found 293.3 and 315.2; calcd for $[M+H]^+$ and $[M+Na]^+$ 293.1 and 315.1, respectively.

A solution of dicyclohexylcarbodiimide (46 mg, 0.22 mmol) in dry DMF (1 ml) was added gradually to a solution of 1-(2,4-dinitrophenyl)-imidazole 4-acetic acid (44 mg, 0.15 mmol) and N-hydroxysuccinimide (21 mg, 0.18 mmol) in anhydrous DMF (1 ml) under nitrogen in an ice bath with stirring. The mixture was stirred for 1 hr at 0 °C,

then overnight at room temperature. The solution was evaporated to dryness and the residue was dissolved in ethyl acetate. The solution was filtered and concentrated by evaporation to give N-hydroxysuccinimide ester of 1-(2,4-dinitrophenyl)-imidazole 4-acetic acid as a brown powder, 70 mg. The activated ester was used without further purification.

5-N-(6-Aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphosphate (**1**) (3.0 $OD_{260\ nm}$, 0.33 μmol) was dissolved in a solution containing distilled water (180 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 9.5) (30 μl) at room temperature. The solution of active ester (3.3 mg, 8.6 μmol)) in dry DMF (90 μl) was added to the solution of **1** and stirred for 20 h at room temperature. The resulting precipitate was removed and 300 μl of 2 M aqueous ammonia was added to the solution and kept overnight at room temperature to de-block the 2,4-dinitrophenyl group. The mixture was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm) with a linear gradient elution of acetonitrile (2.1-51.1 %) in 50 mM triethylammonium acetate (pH 7.2) for 50 min at a flow rate of 1.0 ml/min. The purified **4** was obtained in 43 % yield (1.3 $OD_{260\ nm}$, 0.14 μmol). ESI-Mass (negative mode) *e/z*: found 731.3; calcd for $[M-H]^-$ 731.1.

**5-N-(6-[Urocanyl]aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (5).** A solution of dicyclohexylcarbodiimide (114 mg, 0.55 mmol) in dry DMF (1 ml) was added gradually to a solution of urocanic acid (69mg, 0.50 mmol) and N-Hydroxysuccinimide (63 mg, 0.55 mmol) in anhydrous DMF (2 ml) under nitrogen in an ice bath with stirring. The mixture was stirred for 1 hr at 0 °C, then

overnight at room temperature. The active ester was generated immediately prior to use without further purification. .

5-N-(6-Aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphosphate (**1**) (2.0 $OD_{260\ nm}$, 0.22 μmol) was dissolved in a solution containing distilled water (120 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 9.5) (20 μl) at room temperature. A solution of the active ester in dry DMF (60 μl) was added to the solution of **1** and stirred for 20 h at room temperature. The mixture was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm) with a linear gradient elution of acetonitrile (2.1-58.1 %) in 50 mM triethylammonium acetate (pH 7.2) for 56 min at a flow rate of 1.0 ml/min. The purified **5** was obtained in 96 % yield (5.3 $OD_{260\ nm}$, 0.21 μmol). The yield was calculated from the estimation that the molar absorption coefficient of the product is the sum of that of 5-N-(6-aminohexyl)-carbamoylmethyl-2'-deoxyuridine and urocanic acid. ESI-Mass (negative mode) *e/z*: found 723.1; calcd for $[M-H]^-$ 723.1.

**5-N-(6-[Biotinyl]aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (6).** A solution of dicyclohexylcarbodiimide (77 mg, 0.37 mmol) in dry DMF (2 ml) was added gradually to a solution of biotin (61mg, 0.25 mmol) and N-hydroxysuccinimide (35 mg, 0.30 mmol) in anhydrous DMF (5 ml) under nitrogen at 0 °C with stirring. The mixture was stirred for 1 hr at 0 °C, then overnight at room temperature. The solution was evaporated to dryness and the residue was dissolved in ethyl acetate. The solution was filtered to remove the precipitate and concentrated by evaporation to give N-hydroxysuccinimide ester of biotin as a white powder, 47 mg.

The activated ester was used without further purification, and was generated immediately prior to use.

5-N-(6-Aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphosphate (**1**) (2.0 $OD_{260\ nm}$, 0.22 μmol) was dissolved in a solution containing distilled water (130 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 8.0) (50 μl) at room temperature. A solution of the biotin active ester (0.34 mg, 1.0 μmol) in dry DMF (40 ㎖) was added and stirred for 2 h at room temperature. The mixture was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm) with a linear gradient elution of acetonitrile (2.1- 30.1 %) in 50 mM triethylammonium acetate (pH 7.2) for 28 min at a flow rate of 1.0 ml/min. The purified **6** was obtained in 19 % yield (0.37 $OD_{260\ nm}$, 0.04 μmol). ESI-Mass (negative mode) *e/z*: found 849.3; calcd for $[M-H]^-$ 849.2.

**5-N-(6-[3-(2-Pyridyldithio)propionyl]aminohexyl)carbamoylmethyl-2'-deox yuridine 5'-triphospahte (7).** 5-N-(6-Aminohexyl)carbamoylmethyl-2'- deoxyuridine 5'-triphosphate (**1**) (10 $OD_{260\ nm}$, 1.1 μmol) was dissolved in a solution containing distilled water (200 μl) and 0.2 M sodium phosphate buffer (pH 7.2) (50 μl) at room temperature. A solution of N-hydroxysuccinimide ester of 3-(2-pyridylthio)propionate (0.72 mg, 2.3 μmol) in dry DMF (250 ㎖) was added and stirred for 2 h at room temperature. The mixture was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm) with a linear gradient elution of acetonitrile (2.1-37.1 %) in 50 mM triethylammonium acetate (pH 7.2) for 35 min at a flow rate of 3.0 ml/min. The nucleotide **7** was obtained in 59 % yield (5.9 $OD_{260\ nm}$, 0.64 μmol). The yield was calculated from the estimation that the molar absorption coefficient of the product is the

sum of that of 5-N-(6-aminohexyl)carbamoylmethyl-2'-deoxyuridine and 3-(2-pyridylthio)propionic acid. ESI-Mass (negative mode) *e/z*: found 820.0; calcd for $[M-H]^-$ 820.1.

**5-N-(6-[3-Mercaptopropinonyl]aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (8).** An aqueous solution of dithiothreitol (0.1 M) was added to a solution of 5-N-(6-[3-(2-pyridylthio)propionyl]aminohexyl)carbamoyl-methyl-2'-deoxyuridine 5'-triphospahte (5.0 $OD_{260\ nm}$, 0.54 μmol) in distilled water (100 μl) and the mixture was stirred for 3h at room temperature. The solution was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm) to give the nucleotide **8** in 66 % yield, (3.3 $OD_{260\ nm}$, 0.36 μmol). ESI-Mass (negative mode) *e/z*: found 711.0; calcd for $[M-H]^-$ 711.1.

**5-N-(6-[ε-Trifulouroacetylamidohexyl]aminohexynyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (9).** 5-N-(6-Aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphosphate (**1**) (4.0 $OD_{260\ nm}$, 0.43 μmol) was dissolved in a solution containing distilled water (50 μl) and 0.2 M sodium phosphate buffer (pH 7.2) (50 μl) at room temperature. A solution of N-hydroxysuccinimide ester of *N*-(ε-trifluoroacetylaminohexanoic acid (0.49 mg, 1.5 μmol) in dry DMF (100 μl) was added and stirred for 4 h at room temperature. The mixture was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm). The nucleotide **9** was obtained in 88 % yield (3.5 $OD_{260\ nm}$, 0.38 μmol). ESI-Mass (negative mode) *e/z*: found 832.2; calcd for $[M-H]^-$ 832.2.

**5-N-(6-[Aminohexynyl]aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (10).** A solution of compound **9** (1.5 $OD_{260\ nm}$, 0.16 μmol) in distilled water (50 μl) was treated with 4.0 M aqueous ammonia and stirred for 3 h at room temperature. The solution was concentrated and applied to HPLC on an ODS-silica gel column (4 mm x 250 mm). The nucleotide **10** was obtained in 93 % yield (1.4 $OD_{260\ nm}$, 0.15 μmol). ESI-Mass (negative mode) *e/z*: found 736.4; calcd for $[M-H]^-$ 736.2.

**5-N-(6-Succinylaminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphospahte (11).** 5-N-(6-Aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphosphate (**1**) (2.0 $OD_{260\ nm}$, 0.22 μmol) was dissolved in a solution containing distilled water (160 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 9.5) (20 μl) at room temperature. A solution of succinic anhydride (0.204 mg, 2.0 μmol) in dry DMF (20 μl) was added and stirred for 20 h at room temperature. The mixture was concentrated and purified by HPLC on an ODS-silica gel column (4 mm x 250 mm). The elution was carried out with a linear gradient of acetonitrile (2.1- 30.1 %) in 50 mM triethylammonium acetate (pH 7.2) for 28 min at a flow rate of 1.0 ml/min. The nucleotide **11** was obtained in 70 % yield (1.4 $OD_{260\ nm}$, 0.15 μmol). ESI-Mass (negative mode) *e/z*: found 723.1; calcd for $[M-H]^-$ 723.1.

**5-N-(6-[9-Phenanthrylcarbonyl]aminohexyl)carbamoylmethyl-2'-deoxyuridine 5'-triphosphate (12).** A solution of dicyclohexylcarbodiimide (56 mg, 0.27 mmol) and N-Hydroxysuccinimide (30 mg, 0.26 mmol) in dry DMF (0.9 ml) was added gradually to a solution of 2-carboxy-1,10-phenanhroline (40 mg, 0.18 mmol) in dry

DMF (0.9 ml) under nitrogen in an ice bath with stirring. The mixture was stirred for 1 hr at 0 °C, then overnight at room temperature. The activated ester was used without further purification, and was generated immediately prior to use.

The above solution of the active ester (100 μl) was added to a solution of **1** (4.0 $OD_{260\ nm}$, 0.43 μmol) in distilled water (80 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 9.5) (20 μl) at room temperature with stirring, and the mixture was stirred for 5 h at 37 °C. The mixture was concentrated and subjected to HPLC on an ODS-silica gel column (10 mm x 250 mm). The purified **12** was obtained in 45 % yield (7.7 $OD_{260\ nm}$, 0.20 μmol). The yield was calculated from the estimation that the molar absorption coefficient of the product is the sum of that of 5-N-(6-aminohexyl)-carbamoylmethyl-2'-deoxyuridine and 1,10-phenanthroline-2-carboxylic acid. ESI-Mass (negative mode) *e/z*: found 829.1; calcd for $[M-H]^-$ 8291.

**5-N-(6-[5-(2,9-Dimethylphenanthroline)thiouryl]aminohexyl)carbamoyl-methyl-2'-deoxyuridine 5'-triphosphate (13).** 2,9-Dimethylphenanthroline (416 mg, 1.9 mmol) was dissolved in a solution containing fuming nitric acid (2 ml) and sulfuric acid (3ml) and stirred for 5 h at 95 °C. After cooling, the mixture was neutralized with sodium hydroxide solution and extracted with chloroform. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness to give 5-nitro-2,9-dimethylphenanthroline as yellow foam. The yield was 34 % (165 mg, 0.65 mmol). ESI-Mass (positive mode) *e/z*: found 254.2 and 276.2; calcd for $[M+H]^+$ and $[M+Na]^+$ 254.1 and 276.1, respectively.

5-nitro-2,9-dimethylphenanthroline (160 mg, 0.63 mmol) was hydrogenated in ethanol (5 ml) in the presence of 5 % Pd-carbon (100 mg) under an atmospheric pressure of hydrogen for 14 h at room temperature. After removing the catalyst by filtration, the product was purified by NH-silica gel chromatography using 5 % methanol-dichloromethane as an eluent. 5-Amino-2,9-dimethylphenanthroline was obtained in 58 % yield as yellow foam, 82 mg, 0.37mmol. ESI-Mass (positive mode) *e/z*: found 224.3 and 246.3; calcd for $[M+H]^+$ and $[M+Na]^+$,224.1 and 246.1, respectively.

Thiophosgene (110 μl, 1.4 mmol) was added to a mixture of 5-amino-2,9-dimethylphenanthroline (80 mg, 0.36 mmol) in chloroform (5 ml) and saturated sodium carbonate (2 ml), and the solution was stirred for 4 h at room temperature. The organic layer was washed with water twice, then aqueous sodium chloride and dried over anhydrous sodium sulfate. The solvent was evaporated to dryness to give 2,9-dimethylphenanthroline-5-isothiocyanate as brown foam. This product was used without further purification. ESI-Mass (positive mode) *e/z*: found 266.2; calcd for $[M+H]^+$ 266.1.

A solution of 2,9-dimethylphenanthroline-5-isothiocyanate (1.5 mg, 1.2 μmol) in dry DMF (200 μl) was added to a solution of **1** (4.0 $OD_{260\ nm}$, 0.43 μmol) in distilled water (160 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 9.5) (40 μl) at room temperature with stirring, and the mixture was stirred for 5 h at 37 °C. The mixture was concentrated and subjected to HPLC on an ODS-silica gel column (10 mm x 250 mm). The purified **13** was obtained in 54 % yield (8.8 $OD_{260\ nm}$, 0.23 μmol). The yield was

calculated from the estimation that molar absorption coefficient of the product is the sum of that of 5-N-(6-Aminohexyl)carbamoylmethyl-2'-deoxyuridine and 2,9-dimethylphenanthroline. ESI-Mass (negative mode) *e/z*: found 888.1; calcd for $[M-H]^-$ 888.2.

**5-N-(4-[Fluoresceinthiouryl]aminohexyl)carbamoyl-methyl-2'-deoxyuridine 5'-triphosphate (14).** A solution of fluorescein-4-isothiocyanate (0.4 mg, 1.0 μmol) in dry DMF (20 μl) was added to a solution of **1** (2.0 $OD_{260\ nm}$, 0.22 μmol) in distilled water (130 μl) and 1.0 M sodium hydrogen carbonate buffer (pH 9.5) (50 μl) at room temperature with stirring, and the mixture was stirred for 2 h at 37 °C. The mixture was concentrated and subjected to HPLC on an ODS-silica gel column (4 mm x 250 mm). The purified **14** was obtained in 27 % yield (4.1 $OD_{480\ nm}$, 0.059 μmol). The yield was calculated from the molar absorption coefficient of fluorescein at 480 nm. ESI-Mass (negative mode) *e/z*: found 1012.3; calcd for $[M-H]^-$ 1012.3.

**5-N-(4-[Fluoresceinyl]carbonylaminohexyl)carbamoyl-methyl-2'-deoxyuridine 5'-triphosphate (16).** A solution of hydroxysuccinimide ester of fluorescein 4-carboxylic acid (20mg, 42 μmol) was added to a suspension of **1** (7.3 $OD_{260\ nm}$, 0.85 μmol) in dry DMF (1 mll) containing 2 μl of tri-n-biutylamine and 6 μl of triethylamine at room temperature with stirring, and the mixture was stirred for 5 h at room temperature. The mixture was concentrated and subjected to HPLC on an ODS-silica gel column (4 mm x 250 mm). The purified **16** was obtained in 6 % yield (1.7 $OD_{260\ nm}$). The yield was calculated from the estimation that the molar absorption coefficient of the product is the sum of that of

5-N-(6-aminohexyl)-carbamoylmethyl-2'-deoxyuridine and fluorescein. ESI-Mass (negative mode) *e/z*: found 981.0; calcd for $[M-H]^-$ 981.1