

3,5-Dinitro-1-(4-nitrophenyl)-4-pyridone, A Novel and Convenient Protecting Reagent for Primary Amines¹⁾

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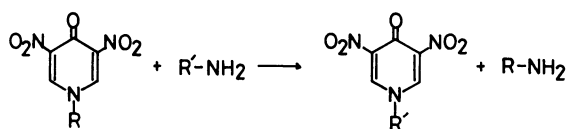
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Crystalline derivatives of the following L-amino acids modified by 3,5-dinitro-4-pyridone have been prepared: glycine, alanine, valine, leucine, isoleucine, phenylalanine, serine, threonine, tyrosine, aspartic acid, asparagine, glutamic acid, glutamine, tryptophan, histidine, arginine, methionine and lysine. The modified L-amino acids (DNPY-L-amino acids) could be purified by recrystallization and were characterized by ¹H-NMR, IR and UV spectral data. The molar rotation of the DNPY-L-amino acids varied from 2 to 100 times those of the parent amino acids. The effectiveness of 3,5-dinitro-1-(4-nitrophenyl)-4-pyridone as an amino-protecting reagent of L-amino acids is described.

We have previously reported that 1-substituted 3,5-dinitro-2- and 4-pyridones reacted with some carbon nucleophiles. In the reaction, a new ring transformation was found by action of alkali salts of β -keto esters and vinyl ethers.²⁾

We have recently shown that the treatment of the 4-pyridone with a primary amine leads to another type of ring transformation reaction. Thus 3,5-dinitro-1-(4-nitrophenyl)-4-pyridone (DNPY³⁾-C₆H₄-NO₂-4) and 1.1 equimolar amounts of propylamine in pyridine medium gave 3,5-dinitro-1-propyl-4-pyridone and 4-nitroaniline in nearly quantitative yields at room temperature.⁴⁾ The reaction involves the nucleophilic attack of the amine in sequence at C-2 and C-6 positions on the 4-pyridone and exchanges of the amine moiety in the pyridone ring, as shown in Scheme 1.



Scheme 1.

The reaction may be referred to the difference in nucleophilicity between the introduced amines and the liberated ones. Therefore the 4-pyridone derivative with an electron-withdrawing substituent at the 1-position should be one of the best protecting reagents for primary amines.

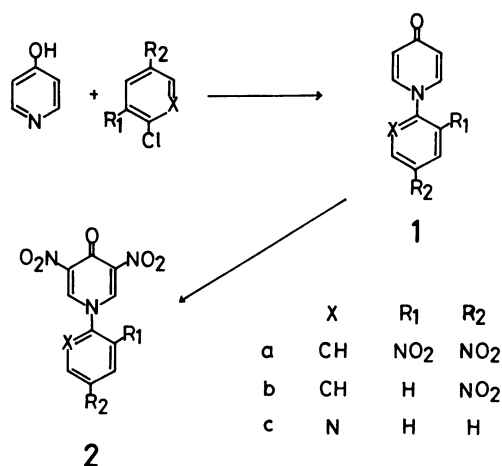
In synthetic organic chemistry, a variety of protective reagents (*e.g.*, urethane-type, acyl-type and alkyl-type), have been developed and utilized.⁵⁾ Here we propose the 1-substituted 3,5-dinitro-4-pyridone as a novel reagent for blocking of the amino function, since the 4-pyridone reacts easily under very mild conditions.

The present paper describes the first application of the reagent to amino acids and the properties of the resulting modified amino acids (DNPY-L-amino acids).

Results and Discussion

Preparation of 1-Substituted 3,5-Dinitro-4-Pyridones. 1-Substituted 3,5-dinitro-4-pyridones (**2a**—**2c**) were

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Scheme 2.

TABLE 1. YIELD OF 1-SUBSTITUTED 3,5-DINITRO-4-PYRIDONES

4-Pyridone	2a	2b	2c
Overall yield ^{a)}	13%	70%	24%

a) Based on 4-pyridinol.

prepared by two step reactions from 4-pyridinol, as shown in Scheme 2.

As has been pointed out, the 4-pyridone with a stronger electron-withdrawing group at the 1-position would be more favorable for a protective reagent. However, the strongest electron-withdrawing group does not always make the pyridone the best reagent for protecting. Tetranitro compound, **2a** is indeed the most electron-deficient, but its precursor **1a** is too electron-poor to give the nitration product **2a** in good yield, as Table 1 shows. Among the three pyridones, **2b** was chosen as the suitable substrate for our purpose in view of the yield obtained and the cost for preparation.

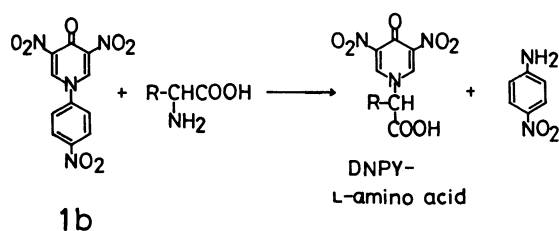
Syntheses of Modified L-Amino Acids (DNPY-L-Amino Acids). In aqueous pyridine, **2b** reacted with eighteen kinds of L-amino acids in high yield, as follows.

The reaction was accomplished in 2—24 h at room

TABLE 2. PHYSICAL PROPERTIES OF THE DNPY-L-AMINO ACIDS (DNPY-A)

DNPY-A A=L-Amino acid	Reaction time (h)	Decomposition Point (°C)	Shape of Crystals	Yield/%
Gly	4.0	220.0	prisms	quant.
Ala	4.0	195.0	needles	quant.
Val	4.0	237.5—239.0	columns	82
Leu	4.0	210.0—212.0	prisms	80
Ile	4.0	240.0—245.0	plates	86
Phe	4.0	105.0—107.0	needles	55
Ser	5.0	184.5—185.5	needles	quant.
Thr	3.0	203.0	needles	75
Tyr	3.0	234.0—235.0	needles	quant.
Asp	5.0	112.0	needles	89
Asn	2.5	130.0—131.5	needles	81
Glu	5.5	218.0—221.0	columns	quant.
Gln	2.0	225.0—226.0	prisms	90
Trp	3.0	191.0—193.0	needles	55
His	3.0	255.0—257.0	plates	quant.
Arg	2.0	175.5—176.0	needles	77
Met	3.0	149.0—151.0	prisms	98
Lys ^{a)}	23.0	190.5—191.5	needles	87

a) Bis-DNPY-L-Lys



Scheme 3.

temperature when all reactants had been dissolved completely.

The solvent was evaporated *in vacuo* and then the residue was extracted with chloroform to remove 4-nitroaniline. The aqueous layer was condensed to give a DNPY L-amino acid. All the L-amino acids except L-lysine could be converted to α -amino-protected compounds. In the case of L-lysine, which had two amino functions, it reacted with an equimolar amount of **2b** to give a mixture of mono-DNPY-L-lysine, bis-DNPY-L-lysine and L-lysine. From L-arginine, on the other hand, α -amino-protected product was only obtained in 77% yield. The results can be explained by the suggestion that the latter's ω -amino function is a part of the amidine structure and is less nucleophilic to **2b** under the conditions used. In the former, however, its ω -amino function reacts easily with **2b** because its pK_a value is 10.28.⁶⁾ Thus treatment of L-lysine with 2 equimolar amounts of **2b** gave bis-DNPY-L-lysine in good yield. In both cases, the reactions under nitrogen atmosphere led to better results.

All of the modified L-amino acids were yellow solids because of the DNPY-protection and could be purified by recrystallization from aqueous ethanol. The DNPY-protection caused the amino function to become tertiarized and the modified L-amino acids to become

less hygroscopic. Thus the DNPY-L-amino acids could be handled more easily. Their structures were determined based upon the spectroscopic data, as follows.

The Spectroscopic Properties of the DNPY-L-amino Acids.

The spectral characteristics of the DNPY-L-amino acids are summarized in Table 3. In the ^1H -NMR spectrum, the aromatic protons on the pyridone ring appear as a sharp singlet at *ca.* $\delta=9.1$. In addition, the α -methine proton of the modified L-amino acid moiety is shifted downfield compared with that of the parent L-amino acid, due to the electron-withdrawing effect of the DNPY group. The infrared spectra of the DNPY-L-amino acids show the stretching vibration of the pyridone carbonyl at *ca.* 1670 cm^{-1} characteristically. Furthermore, the asymmetrical and the symmetrical stretching vibration of the nitro group are present at about 1510 and 1340 cm^{-1} , respectively.

In the ultraviolet spectrum, the absorption identified as the $\pi\text{-}\pi^*$ transition of the pyridone ring appears at *ca.* 340 nm with $\epsilon=\text{ca. } 4.5\times 10^3$ characteristically.

The Optical Property of The DNPY-L-amino Acids. The values of the optical rotation have been used as a quantification of the modified amino acids, so those of the DNPY-L-amino acids determined in DMF are listed in Table 4.

The molar rotation of the DNPY-L-amino acids, $[\text{M}]_D$ are in general higher and in some cases very much higher than those of the parent L-amino acids. Furthermore, the values are comparable with or superior to those of well-known DNP-L-amino acids, of which the molar rotations vary from 2 to 100 times those of the parent amino acids.⁷⁾ The results may permit the identification and the optical characterization of a small quantity of an amino acid enantiomorph, so the DNPY group would be useful for amino-protection.

Removal of the DNPY Group. For the removal

TABLE 3. SPECTROSCOPIC PROPERTIES OF THE DNPY-L-AMINO ACIDS (DNPY-A)

DNPY-A A=L-Amino acid	IR $\bar{\nu}/\text{cm}^{-1}$		$^1\text{H-NMR}$ $\delta/\text{ppm}^{1)}$		UV ²⁾	
	$\nu_{\text{C=O}}^{3)}$	ν_{NO_2}	$\text{H}_2 \text{ \& \text{H}}_6$	$\alpha\text{-methine}$	$\lambda_{\text{max}}/\text{nm}$	$\epsilon(\text{at max}) \times 10^3$
Gly	1674	1510, 1345	9.13	—	342	6.92
Ala	1673	1510, 1342	9.15	5.34(q)	341	4.23
Val	1675	1512, 1346	9.12	4.92(d)	342	4.65
Leu	1676	1512, 1345	9.16	5.23(dd)	340	4.17
Ile	1670	1512, 1345	9.13	4.97(d)	342	5.16
Phe	1672	1510, 1340	9.10	5.63(t)	342	2.74
Ser	1675	1505, 1336	9.14	5.36(dd)	341	4.83
Thr	1675	1510, 1340	9.09	5.22(d)	340	4.67
Tyr	1670	1508, 1335	9.03	5.53(t)	340	3.96
Asp	1670	1510, 1340	9.24	5.63(t)	340	3.72
Asn	1670	1513, 1340	9.19	5.46(t)	340	4.45
Glu	1677	1510, 1340	9.15	5.20(m)	342	4.30
Gln	1677	1510, 1340	9.11	5.24(t)	354	4.17 ⁴⁾
Trp	1670	1510, 1325	9.01	5.62(t)	340	4.64
His	1680	1510, 1325	9.04	5.35(t)	—	— ⁵⁾
Arg	1673	1510, 1335	9.12	5.30(m) ⁶⁾	346 ⁷⁾	3.97
Met	1665	1526, 1352	9.15	5.30(m)	339	4.17
Lys ⁸⁾	1688 1678	1511, 1320	9.15 9.11	5.25(t)	358	8.10 ⁴⁾

1) In DMSO- d_6 . 2) In EtOH. 3) On pyridone ring. 4) In DMF. 5) Slightly soluble in every solvent. 6) In CF_3COOD . 7) In 6 M-HCl (1 M = 1 mol dm^{-3}). 8) Bis-DNPY-L-Lys.

TABLE 4. OPTICAL PROPERTIES OF THE DNPY-L-AMINO ACIDS (DNPY-A)

DNPY-A A=L-Amino acid	Molecular formula	Found (%)			Calcd (%)			$[\alpha]_D^{25}$ in DMF	$[M]_D$	$[M]_D$ of parent amino acid ^{b)}
		C	H	N	C	H	N			
Gly	$\text{C}_7\text{H}_5\text{N}_3\text{O}_7$	34.82	2.09	17.50	34.58	2.07	17.28	—	—	—
Ala	$\text{C}_8\text{H}_7\text{N}_3\text{O}_7$	37.32	2.73	16.46	37.36	2.74	16.34	−26.2°	−67.4°	+13.0°
Val	$\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_7$	42.07	3.83	14.80	42.11	3.89	14.73	−127.5°	−269.2°	+33.7°
Leu	$\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_7$	44.15	4.35	14.07	44.15	4.38	14.04	−53.5°	−118.5°	+30.0°
Ile	$\text{C}_{11}\text{H}_{13}\text{N}_3\text{O}_7$	43.94	4.19	14.12	44.15	4.38	14.04	−114.0°	−252.5°	+53.1°
Phe	$\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_7$	50.70	3.50	12.39	50.45	3.33	12.61	−150.0°	−370.0°	−7.4°
Ser	$\text{C}_8\text{H}_7\text{N}_3\text{O}_8$	35.20	2.60	15.49	35.17	2.58	15.38	−0.87°	−1.8°	+15.9°
Thr	$\text{C}_8\text{H}_9\text{N}_3\text{O}_8$	37.48	3.09	14.64	37.64	3.16	14.63	+25.0°	+53.1°	−17.9°
Tyr	$\text{C}_{14}\text{H}_{11}\text{N}_3\text{O}_8$	48.27	3.03	12.02	48.14	3.17	12.03	−208.0°	−537.7°	−21.4°
Asp	$\text{C}_9\text{H}_7\text{N}_3\text{O}_9$	35.44	2.43	13.87	35.89	2.34	13.95	−69.5°	−154.9°	+34.9°
Asn	$\text{C}_9\text{H}_8\text{N}_4\text{O}_8$	35.91	2.88	18.37	36.01	2.69	18.67	+30.5°	+67.8°	+45.3° ^{e)}
Glu	$\text{C}_{10}\text{H}_9\text{N}_3\text{O}_9$	42.02	3.34	13.66	42.20	3.26	13.78 ^{a)}	−71.5°	−166.8°	+47.4°
Gln	$\text{C}_{10}\text{H}_{10}\text{N}_4\text{O}_8$	38.34	3.21	17.83	38.22	3.21	17.83	−83.5°	−194.2°	+57.3° ^{f)}
Trp	$\text{C}_{16}\text{H}_{12}\text{N}_4\text{O}_7$	51.38	3.22	14.84	51.62	3.25	15.05	−190.8°	−525.7°	+5.7°
Arg	$\text{C}_{11}\text{H}_{14}\text{N}_6\text{O}_7$	38.47	4.10	24.67	38.60	4.12	24.56 ^{b)}	−3.5°	−8.9°	+48.0°
Met	$\text{C}_{10}\text{H}_{11}\text{N}_3\text{O}_7\text{S}$	37.76	3.53	13.10	37.86	3.49	13.24	−61.5°	−143.2°	+36.0°
Lys ^{c)}	$\text{C}_{16}\text{H}_{14}\text{N}_6\text{O}_{12}$	39.70	3.08	17.41	39.84	2.93	17.43	−52.3°	−186.7°	+38.0°
His	$\text{C}_{11}\text{H}_9\text{N}_5\text{O}_7$	40.60	2.80	21.59	40.88	2.81	21.67	— ^{d)}	—	+19.2°

a) Calcd for $\text{C}_{10}\text{H}_9\text{N}_3\text{O}_9 \cdot 1/2\text{C}_5\text{H}_5\text{N}$. b) In 5 M-HCl. c) Bis-DNPY-L-Lys. d) Slightly soluble in every solvent. e) In 3 M-HCl. f) In 1 M-HCl.

of the DNPY group from the modified L-amino acid, a deblocking reagent must be more nucleophilic than the corresponding L-amino acid, of which the α -amino function generally has a pK_a value of 9.1–9.8.⁶⁾ In addition, on the deblocking reaction, the liberated L-amino acid has to be easily separated from the DNPY-derivative formed. Thus hexylamine, whose pK_a

value was 10.64⁸⁾ was selected as a deblocking reagent. The removal reaction also proceeded in aqueous pyridine medium at room temperature for 0.5–2.5 h similar to the blocking process. The liberated L-amino acid was recovered in good yield after the same post-treatment as used for the blocking procedure. The results are shown in Table 5, along with the specific

TABLE 5. YIELD AND SPECIFIC ROTATION OF RECOVERED L-AMINO ACIDS

L-Amino acid	Yield/%	$[\alpha]_D^{25}$ in 5 M-HCl (lit.) ^{a)}
Ala	91.7	+14.9° (+14.7°)
Leu	97.0	+14.7° (+14.7°)
Phe	85.7	-4.5° (-4.5°)
Ser	90.9	+14.9° (+15.1°)
Thr	83.8	-14.6° (-15.1°)
Tyr	88.3	-11.3° (-11.8°)
Glu	84.0	+32.3° (+32.2°)
Asn	96.2	+34.8° (+34.3°)
His	92.0	+12.4° (+12.4°)
Met	85.1	+25.3° (+24.1°)

a) Reference 6).

rotation.

No change in specific rotation of the L-amino acid throughout the DNPY-modification was found, within an uncertainty of about four percent. The results indicate that racemization does not occur; this is an important advantage of the DNPY-modification.

Subsequently, the DNPY-modification is very utilizable for amino-protection of amino acids in following aspects.

- (1) 3,5-Dinitro-1-(4-nitrophenyl)-4-pyridone, the protecting reagent is easily prepared from pyridine with a sequence of only three reactions.
- (2) The blocking and deblocking processes proceed under very mild conditions, in aqueous pyridine and at room temperature with stirring.
- (3) The tertiarization by DNPY-protection makes an amino acid less hygroscopic and the modified L-amino acid can be handled more easily.
- (4) The DNPY-L-amino acids are identified very easily by the spectroscopic data of the characteristic DNPY group.
- (5) No racemization takes place throughout the modification.

The DNPY-modification tertiarized the nitrogen of the α -amino group on every L-amino acids like phthaloyl-masking.

The latter, however, has not been applied to tryptophan.⁹⁾ Furthermore, its blocking-deblocking processes are more complicated and drastic than the DNPY-method. In these aspects, the DNPY-protecting is superior to the phthaloyl-modification.

Further work is in progress, and the data of esterification and of peptide synthesis with the DNPY-L-amino acid will be reported elsewhere.

Experimental

Measurements. All melting points were determined on a Yanako Melting Point Apparatus and are uncorrected. ¹H-NMR, IR and UV spectra were obtained on a HITACHI R-20B High Resolution Spectrometer, a HITACHI 225 Grating Infrared Spectrophotometer, and a HITACHI EPS-3 Recording Spectrophotometer, respectively. Optical rotation was determined on a Jasco DIP-180 automatic polarimeter.

3,5-Dinitro-(4-nitrophenyl)-4-pyridone (2b). Two and a half grams of sodium metal was dissolved in 100 ml of abso-

lute ethanol and then 10 g of 4-pyridinol was added gradually. After 30 min, the mixture was evaporated and the residue was dissolved in 50 ml of DMF.

To the solution, a DMF (50 ml) solution of 18 g of 1-chloro-4-nitrobenzene was added dropwise and the resulting mixture was heated at 150 °C for 10 h. After cooling, the mixture was evaporated *in vacuo*, and water was added to the residue. The aqueous solution was acidified till pH 5. Yellow crystalline precipitates were collected and recrystallized from water to give 1-(4-nitrophenyl)-4-pyridone (**1b**) quantitatively, mp 191–192 °C; IR (as Nujol mulls): 1675 cm⁻¹ (C=O), 1530, 1350 (NO₂); ¹H-NMR (DMSO-*d*₆): δ =6.29 (2H, d, *J*=8 Hz, β -protons of pyridone ring), 8.13 (2H, d, *J*=8 Hz, α -protons of pyridone ring), and 7.86 and 8.37 (4H, AA'BB', *J*=9 Hz, aromatic protons of 1,4-disubstituted benzene ring).

One gram of 1-(4-nitrophenyl)-4-pyridone (**1b**) was dissolved in 20 ml of concentrated sulfuric acid (*d*=1.84), and then 5.8g of fuming nitric acid (*d*=1.52) was added gradually with ice cooling. The mixture was poured over crushed ice. The yellow crystalline solid was collected and washed with cold water. The solid was recrystallized from aqueous acetic acid (*ca.* 50%) to give **2b** as yellow needles in 70% yield; mp 264–265 °C; IR (as Nujol mulls): 1680 cm⁻¹ (C=O), 1600, 1540, 1500, 1345, 850; ¹H-NMR (DMSO-*d*₆): δ =9.36 (2H, sharp s, α -protons of pyridone ring), and 8.47 and 8.06 (4H, AA'BB', *J*=9 Hz, aromatic protons of 1,4-disubstituted benzene ring); Found: C, 42.84; H, 2.15; N, 18.39%. Calcd for C₁₁H₆N₄O₇: C, 43.15; H, 1.98; N, 18.30%.

The other 1-substituted 3,5-dinitro-4-pyridones, **2a** and **2c** were prepared in a way similar to that used for **2b**.

L-Amino acids were supplied by Wako Pure Chemical Industries, Ltd. The protecting reaction by **2b** was carried out as follows.

In a round-bottomed flask were placed **2b** (2.0 mmol), L-amino acid (2.2 mmol) and 60 ml of water-pyridine (3:1). The mixture was stirred with a magnetic stirrer at room temperature. (In the cases of L-Arg and L-Lys, under nitrogen stream.) After all reactants had dissolved, the solvent was removed below 30 °C *in vacuo*. A yellow residue was obtained, to which water was added. The resulting mixture was extracted with chloroform to remove 4-nitroaniline. The aqueous layer was acidified with dilute hydrochloric acid till pH 1–2 and then evaporated under reduced pressure to give a yellow crystalline solid. The crude product was collected and washed with water, and recrystallized from aqueous ethanol.

The purified DNPY-L-amino acid was dried over phosphorus pentaoxide at 100 °C for 5–6 h *in vacuo*.

Deblocking Reaction of The DNPY-L-amino Acid. In a round-bottomed flask were placed DNPY-L-Ala (1 mmol), hexylamine (1.1 mmol) and 15 ml of pyridine. The mixture was stirred at room temperature for 1.5 h. The solvent was evaporated *in vacuo* and water was added to the residue. The mixture was extracted with chloroform. The water layer was neutralized and condensed to give L-Ala in 92% yield. After dryness, L-Ala was identified by the spectroscopic data and its specific rotation. The organic layer was evaporated to give 1-hexyl-3,5-dinitro-4-pyridone⁴⁾ quantitatively, which was identified by comparing with an authentic sample. The other DNPY-L-amino acids were treated similarly to give the parent L-amino acids.

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