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CD SPECTRA OF DNP DERIVATIVES OF AROMATIC α-AMINO ACIDS AND RELATED COMPOUNDS

DNP-AROMATIC RULE AS A METHOD FOR DETERMINING ABSOLUTE CONFIGURATION OF CHIRAL AMINES OF RCH(NH$_2$)XAr TYPE

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Abstract—Introduction of an aromatic group to the side-chain functional group or to the α-carboxyl group of aliphatic DNP-α-amino acids gives compounds which can be regarded as analogs of aromatic DNP-α-amino acids. DNP-aromatic rule[1] has been proved applicable to these compounds and also to the derivatives of DNP-phenylalanine homologs in which the carboxyl group is modified variously. By summarizing the compiled data extension of the DNP-aromatic rule to the DNP-derivatives of chiral amines with a general formula R–CH(NH$_2$)-X-Ar is proposed.

An extensive study on the CD spectra of N-(2,4-dinitrophenyl) (=DNP) derivatives of α-amino acids have been undertaken.[1–4] According to their spectral pattern the DNP derivatives are classified into four groups (Fig. 1): (i) di-DNP derivatives of α, ω-diamino acids, (ii) DNP derivatives of α-amino acids having an aromatic group on their side chain, (iii) DNP derivatives of cyclic α-amino acids and (iv) DNP derivatives of other α-amino acids which do not fall under (i)–(iii). While CD spectra of group (iv) compounds have very weak intensity above 300 nm ($-2000 < [\theta]_{405}^{max} < +2000$)†, each of the other groups exhibits characteristic pattern of CD spectra of much higher intensity in this region. Several rules relating the sign of the Cotton effect near 400 nm to the absolute configuration of parent amino acids were found for each of the groups, (i),[3] (ii),[1] and (iii).[4] The rules are applicable not only to α-amino acids but also to other chiral amino compounds with the similar structural features. In this paper detailed study on "DNP-aromatic rule"[1] and its applicability to chiral amines expressed by a general formula RCH(NH$_2$)XAr, where X means a chain containing no chiral center, are described.

DNP-aromatic rule relates the absolute configuration of some primary amino compounds to the sign of the Cotton effect at the longest wavelength band near 400 nm of their DNP derivatives ($[\theta]_{400}^{max} < 0$ for L- and > 0 for D-configuration). The rule is based on the finding that all the DNP derivatives of common aromatic L-α-amino acids (**1a**–**1f**) and of L-phenylalanine homologs (**2a**–**2e**) show characteristic pattern of CD spectra above 300 nm (Tables 1 and 2).[1] Their two CD bands near 400 and 340 nm are assigned to charge transfer transitions, $^{+}RHN{=}C_1 \rightarrow C_2{=}NO_2^{-}$ and $^{+}RHN{=}C_1 \rightarrow C_4{=}NO_2^{-}$, of the 2,4-dinitroanilino chromophore, respectively.[5] The rule was verified by further examples of DNP-L-α-amino acids (**1g**–**1l**) having various aromatic groups in the β-position as shown in Table 1. The DNP derivative (**2f**) of L-5-(*p*-methoxyphenyl)-2-aminopeptanoic acid, a constituent amino acid of a phyto-toxic depsipeptide produced by *Alternaria mali*,[6] also exhibits negative maximum near 400 nm, demonstrating applicability of the rule to determination of the absolute configuration of uncommon aromatic amino acids, which sometimes occur in antibiotics and other biologically important peptides. H. Inouye *et al.*[7] isolated an unusual amino acid, β-(5-hydroxy-2- pyridyl)-alanine, from a fermentation broth of a *Streptomyces sp.* and assigned L-configuration based on pH shift of rotation. Our result of the DNP derivative (**1l**) confirms their assignment.

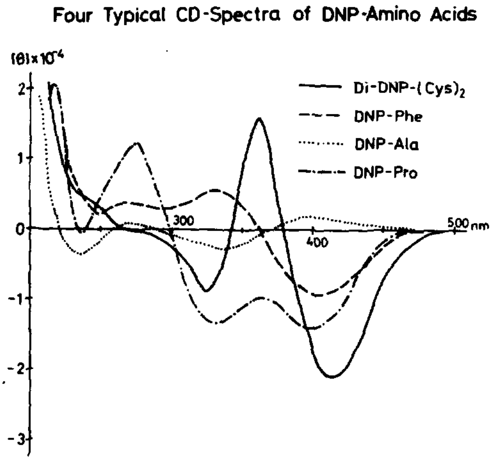
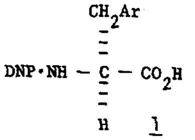
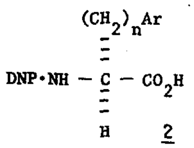


Fig. 1. Four typical CD spectra of DNP-amino acids.



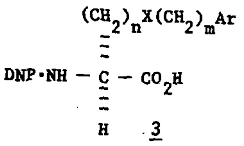
$$\mathrm{DNP{\cdot}NH - \underset{\underset{\displaystyle H}{\vdots}}{\overset{\overset{\displaystyle CH_2Ar}{\vdots}}{C}} - CO_2H}$$

1



$$\mathrm{DNP{\cdot}NH - \underset{\underset{\displaystyle H}{\vdots}}{\overset{\overset{\displaystyle (CH_2)_nAr}{\vdots}}{C}} - CO_2H}$$

2



$$\mathrm{DNP{\cdot}NH - \underset{\underset{\displaystyle H}{\vdots}}{\overset{\overset{\displaystyle (CH_2)_nX(CH_2)_mAr}{\vdots}}{C}} - CO_2H}$$

3

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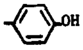
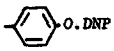
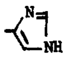
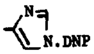
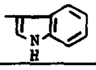
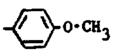
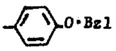
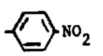
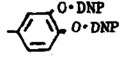
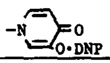
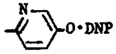
†The only exception is DNP-L-methionine ($[\theta]_{405}^{max} = -3600$). This may be related to the larger $[\theta]_{400}^{max}$ value of **3c** and the smaller value of **7h** compared with those of the corresponding other aliphatic amino acids.

Table 1. CD spectral data of N-DNP-β-aryl-L-alanines with a general formula 1

Compounds	Ar=	λ (nm) / [θ] 10^{-2}								
1a		410	363	334	(296)	270	(254)	212		
		-91	0	+74	+21	+46	+23	+590		
1b		410	372	340	(290)	275	(260)	234	(228)	207
		-90	0	+97	+27	+33	+23	+174	+124	+540
1c		410	372	339	(296)	276	(255)			
		-108	0	+204	+21	+54	+34			
1d		410	368	337	(260)	215				
		-54	0	+51	+7	+200				
1e		416	382	348	(295)	274	250	210		
		-81	0	+146	+24	+51	0	+220		
1f		424	372	344	290	271	262	*250	223	
		-121	0	+62	0	-47	0	+49	+460	
1g		414	369	338	(290)	275	(260)			
		-97	0	+103	+27	+39	+25			
1h		412	370	338	(290)	*270	235	(226)		
		-121	0	+136	+44	+58	+307	+250		
1i		402	356	333	(292)	270	249	236	230	208
		-145	0	+214	+62	+100	0	-67	0	+350
1j		406	370	332	*280	265				
		-108	0	+267	+22	0				
1k		400	369	331	(288)	272	256	244	232	208
		-139	0	+323	+36	+72	0	-52	0	+330
1ℓ		407	367	332	(295)	265	(245)	215		
		-54	0	+90	+16	+42	+11	+170		

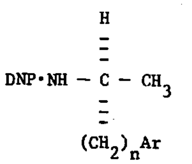
The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity value ([θ]×10^{-2}) at the wavelengths.

Table 2. CD spectral data of the homologs of DNP-L-phenylalanines with a general formula 2

Compounds	n=	Ar=	λ(nm) / [θ]×10^{-2}							
2a	0	C_6H_5	404	352	330	289	276	265	225	
			-118	0	+50	0	-35	0	+710	
2b	2	C_6H_5	414	350	330	295	*260			
			-62	0	+11	0	+3			
2c	3	C_6H_5	415	355	330	(295)	270	250	238	231
			-42	0	+23	+11	+21	0	-12	0
2d	4	C_6H_5	410	362	333	265	237	229		
			-35	0	+24	0	-19	0		
2e	5	C_6H_5	415	362	336	290	270	247	234	226
			-25	0	+17	0	+5	0	-25	0
2f	3	C_6H_4OMe(p)	405	352	*325	270	(250)	207		
			-64	0	+25	+48	+25	+380		

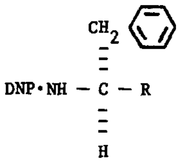
The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity value ([θ]×10^{-2}) at the wavelengths.

It is expected that DNP-aromatic rule can be applied to α-amino acids having a functional group on their side chain by suitable modification. It is quite common to protect a side chain functional group with a benzyl or a benzyloxycarbonyl group. DNP derivatives of such protected amino acids can be regarded as analogs of DNP-phenylalanine homologs (**2**) in which one of the methylene groups is replaced by a hetero atom (0 or S) or a group (–CO_2–, –$NHCO_2$–, etc.). As expected all the derivatives **3a**–**3f** carrying an aromatic group on their side chain functional group exhibited negative Cotton effect near 400 nm (Table 3) reflecting their L-configuration.



$$\mathrm{DNP{\cdot}NH-\overset{\displaystyle H}{\underset{\displaystyle (CH_2)_nAr}{C}}-CH_3}$$

4



5

In order to know whether the carboxyl group is essential for DNP-aromatic rule to be valid, some analogs of DNP derivatives of aromatic α-amino acids, in which the carboxyl group was replaced by other groups, were prepared and their CD spectra were examined. DNP derivatives of chiral aralkylamines of S-configuration, **4a** and **4b**,[8] are enantiomers of analogs of **2a** and **2b**, respectively, having a Me in place of a carboxyl group. CD spectral patterns of **4a** and **4b** were enantiomeric to those of **2a** and **2b**, respectively, indicating that replacement of the carboxyl with a Me group has no significant effect upon their CD spectra. CD spectra of DNP derivatives of S-configuration **4c**–**4e** also exhibited positive Cotton effect near 400 nm (Table 4). Recently, some additional compounds of this type were subjected CD measurement by Bettoni *et al.*,[9] and their results support the rule. It was shown that ionization of the carboxyl group of **1a** and **2a**–**2e** by converting them into dicyclohexylammonium salts also does not cause any marked change of the spectra (Table 5). All the

Table 3. CD spectral data of DNP-L-α-amino acids having a side chain functional group substituted with an aromatic group (general formula 3)

Compounds	n=	X=	m=	Ar=	λ(nm) [θ]×10^{-2}							
3a	1	OCO	0	C_6H_5	408	374	340	(290)	238	219		
					-34	0	+77	+20	+117	0		
3b	1	O	1	C_6H_5	410	360	340	315	280	255	236	227
					-22	0	+5	0	+23	0	-34	0
3c	1	S	1	C_6H_5	402	337	280	260	248	240		
					-98	0	+47	0	-24	0		
3d	1	CO_2	1	C_6H_5	400	340	330	310	275	260	246	233
					-29	0	+5	0	+30	0	-15	0
3e	1	CONH	0	C_6H_4-NO_2(p)	400	368	340	313	304	293	277	259
					-92	0	+250	0	-28	0	+36	0
3f	2	CO_2	1	C_6H_5	400	310	275	252	245	236		
					-40	0	+30	0	-20	0		
3g	2	CONH	0	C_6H_4-NO_2(p)	402	371	342	319	306	292	275	260
					-108	0	+202	0	-20	0	+13	0
					250	242						
					-18	0						
3h	2	OCO	0	C_6H_5	405	360	340	305	*260			
					-20	0	+9	0	+13			
3i	3	$NHCO_2$	1	C_6H_5	410	345	330	305	268	250	239	230
					-36	0	+10	0	+21	0	-22	0
3j	3	NHCO	0	C_6H_5	405	341	324	(308)	264	235		
					-59	0	+11	+3	+75	0		
3k	4	$NHCO_2$	1	C_6H_5	410	357	330	(300)	270	255	236	228
					-33	0	+14	+3	+11	0	-27	0
3ℓ	4	NHCO	0	C_6H_5	408	352	332	300	265	244		
					-42	0	+22	0	+28	0		

The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity values ([θ]×10^{-2}) at the wavelengths.

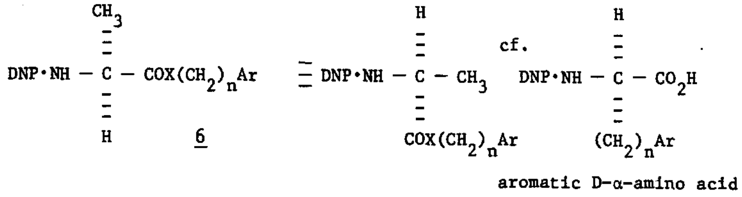
Table 4. CD spectral data of N-DNP-phenethylamines α-substituted with an aromatic group (general formula 4)

Compounds	n=	Ar=	λ(nm) $[\theta]\times 10^{-2}$							
<u>4a</u>	0	$-C_6H_5$	406	352	327	294	275	266	*250	227
			+162	0	-52	0	+55	0	-79	-360
<u>4b</u>	2	$-C_6H_5$	413	340	*325	280	(265)			
			+83	0	-8	-10	-5			
<u>4c</u>	0	$-CH{=}CHC_6H_5$	408	365	335	(290)	255	238	229	216
			+226	0	-216	-67	-626	0	-130	0
			212	208						
			-130	0						
<u>4d</u>	1	$-OCOC_6H_5$	400	347	325	(300)	238			
			+61	0	-23	-9	-170			
<u>4e</u>**	1	$-NHCOC_6H_5$	409	(370)	300	(372)	238			
			+97	+70	0	-45	-166			

The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity values ($[\theta]\times 10^{-2}$) at the wavelengths.

** Actual measurement was carried out using the enantiomer.

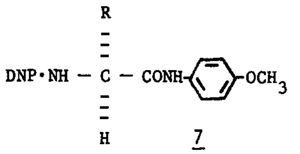
derivatives (**5a–5g**) of the DNP-phenylalanine (**1a**), in which the carboxyl group is modified, showed negative maximum at the longest wavelength, too (Table 6).



$$\mathrm{DNP{\cdot}NH-\overset{CH_3}{\underset{H}{C}}-COX(CH_2)_nAr} \equiv \mathrm{DNP{\cdot}NH-\overset{H}{\underset{COX(CH_2)_nAr}{C}}-CH_3} \quad \text{cf.} \quad \mathrm{DNP{\cdot}NH-\overset{H}{\underset{(CH_2)_nAr}{C}}-CO_2H}$$

<u>6</u>

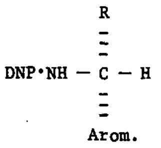
aromatic D-α-amino acid



$$\mathrm{DNP{\cdot}NH-\overset{R}{\underset{H}{C}}-CONH-C_6H_4-OCH_3}$$

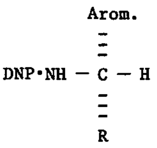
<u>7</u>

On the basis of the results summarized in Table 3 and 4, it is anticipated that the rule could be applied to DNP-α-amino acids having no aromatic side chain by introducing an aromatic substituent on their carboxyl group. Thus, derivatives (**6**) of DNP-L-alanine, whose carboxyl group was converted to an amide or an ester having aromatic ring, were prepared. They can be regarded as analogs of the DNP derivatives of aromatic D-α-amino acids; i.e. the aromatic ester or amide group and the Me group of **6** correspond to the aromatic side chain and the carboxyl group, respectively, of an aromatic α-amino acid with D-configuration. Compounds **6a–6k** showed positive Cotton effect at the longest wavelength, as expected (Table 7). All the *p*-methoxyanilides (**7**) of DNP-L-α-amino acids having various non-aromatic side chain listed on Table 8 and **6b** showed much the same spectra indicating that the CD spectra are due to interaction between the two chromophores and hardly affected by variation of the side chain group R.[2] Ease of preparation as well as large molecular ellipticity ($[\theta] \simeq 10^4$) secures usefulness of these derivatives for determining absolute configuration of newly found amino acids from natural products.



$$\mathrm{DNP{\cdot}NH-\overset{R}{\underset{Arom.}{C}}-H}$$

<u>8a</u>



$$\mathrm{DNP{\cdot}NH-\overset{Arom.}{\underset{R}{C}}-H}$$

<u>8b</u>

All these CD data presented in Tables 1–8 demonstrate the applicability of DNP-aromatic rule to a wide variety of chiral primary amines. Some of the typical CD spectra were reproduced in Fig. 2. The absolute configuration of chiral amines represented in the general formula, **8a** and **8b**, are related to the sign of Cotton effect of their DNP derivatives near 400 nm without any exception: $[\theta]^{max}_{400} <$

Table 5. CD spectral data of dicyclohexylammonium salts of DNP-L-phenylalanine homologs (general formula 2)

Compounds	n=	λ (nm) / $[\theta]\times10^{-2}$								
2a	0	411	356	330	291	275	268	224	(210)	
		-128	0	+54	0	-40	0	+740	+350	
1a	1	425	371	338	(293)	275	(258)	243	(233)	
		-76	0	+71	+20	+42	+11	+63	+31	
2b	2	420	356	330	275	210				
		-60	0	+20	0	+400				
2c	3	415	368	330	*272	258	245	239	231	224
		-34	0	+30	+13	0	+9	0	-32	0
2d	4	418	372	335	275	258	245	230	222	205
		-30	0	+31	0	-23	0	-50	0	+280
2e	5	420	377	330	288	258	246	228	221	
		-20	0	+27	0	-13	0	-67	0	

The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity values ($[\theta]\times10^{-2}$) at the wavelengths.

Table 6. CD spectral data of DNP-L- phenylalanine derivatives modified at the carboxyl group (general formula 5)

Compounds	R=	λ (nm) / $[\theta]\times10^{-2}$								
5a	$CO_2C_2H_5$	405	361	332	(295)	268	244	213		
		-72	0	+57	+22	+41	0	+460		
5b	$CONH_2$	410	360	327	(290)	266	240	212		
		-60	0	+68	+18	+56	0	+380		
5c	$CON(CH_3)_2$	412	350	328	(300)	270	(252)	237	(230)	
		-80	0	+21	+6	+46	+20	+82	+49	
5d	$CONHCH_2CO_2H$	414	368	325	(290)	268	255	234	226	
		-30	0	+53	+16	+40	0	-77	0	
5e	CONR'CONHR'	408	368	334	(295)	265	(247)	240	(228)	
	$(R'=C_6H_{11})$	-46	0	+57	+17	+54	+28	+32	+12	
5f	CH_2OH	418	*375	331	*320	276	(250)	232	(229)	213
		-108	-80	0	+14	+53	+21	+89	+62	+280

The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipitcity values ($[\theta]\times10^{-2}$) at the wavelengths.

0 for **8a** and >0 for **8b**. Here R is an alkyl or other group containing no aromatic chromophore, while Arom. is a group carrying aromatic chromophore and can be expressed as $-(CH_2)_nX(CH_2)_mAr$, where X is a hetero atom or a non-methylene group such as –COO–, –NHCOO–, etc.

It is interesting to note that CD spectra of di-DNP derivatives of L-α,ω-diamino acids, which show negative maximum at the longest wavelength and was explained by exciton chirality theory,[3] are also consistent with the DNP-aromatic rule regarding the ω-DNP group as an aromatic chromophore. Violation of the rule could occur by introducing another chiral center into the Arom. branch of the formula **8**, as is the case of N-DNP-O-benzoyl-L-threonine ($[\theta]_{410}^{max} = +3400$), which should be kept in mind when the rule is applied to complex molecules.

Though CD data presented above are measured in MeOH with the concentration of 5×10^{-5} to 5×10^{-4} M, the sign of Cotton effect is independent of the condition of CD measurements. In order to prove this point, CD spectra of **1a** were measured under various conditions. As shown in Fig. 3 and in Table 9, spectra pattern of **1a** is not affected by variation of concentration nor of the solvent used. At lower temperature markedly amplified spectra were obtained (Fig. 4) indicating that the conformers responsible for the characteristic CD spectrum of **1a** would be thermodynamically stable ones.

Table 7. CD spectral data of DNP-L-alanyl amides and esters with an aromatic or an aralkyl substituent (general formula **6**)

Compounds	X=	n=	Ar=	λ(nm) / $[\theta]\times10^{-2}$								
6a	NH	0	C_6H_5	395	352	329	(285)	246	233	223	212	
				+93	0	-60	-29	-296	0	+124	0	
6b	NH	0	C_6H_4OMe(p)	397	356	330	(293)	255	*244	235	228	215
				+129	0	-92	-33	-240	-208	0	+120	0
6c	NH	0	$C_6H_4NMe_2$(p)	395	358	327	(292)	276	255	252	249	239
				+122	0	-153	-61	-100	0	+2	0	-40
6d	NH	0	$C_6H_4NO_2$(p)	392	362	335	306	301	288	283	278	270
				+169	0	-338	0	+22	0	-12	0	+25
				263	255	(249)	233	224	216	(211)		
				0	-33	-28	-62	0	+87	+69		
6e	NH	0	β-Naphthyl	397	349	333	(292)	253	*247	237		
				+146	0	-205	-38	-560	-520	0		
6f	NH	1	C_6H_5	390	323	314	(305)	280	(269)	230	210	
				+85	0	-4	-2	-31	-20	-280	0	
6g	NH	2	C_6H_5	392	329	*320	267	(265)	231	212		
				+84	0	-4	-40	-29	-236	0		
6h	O	0	C_6H_5	394	357	326	*274	262	234			
				+65	0	-68	-6	0	-157			
6i	O	1	C_6H_5	395	348	327	(286)	276	(265)	235		
				+56	0	-25	-6	-9	-1	-123		
6j	O	2	C_6H_5	400	358	327	(290)	281	268	263	260	234
				+45	0	-37	-16	-18	0	+2	0	-100
6k	O	3	C_6H_5	396	356	327	*284	272	265	260	235	
				+38	0	-35	-4	0	+4	0	-96	

The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity values ($[\theta]\times10^{-2}$) at the wavelengths.

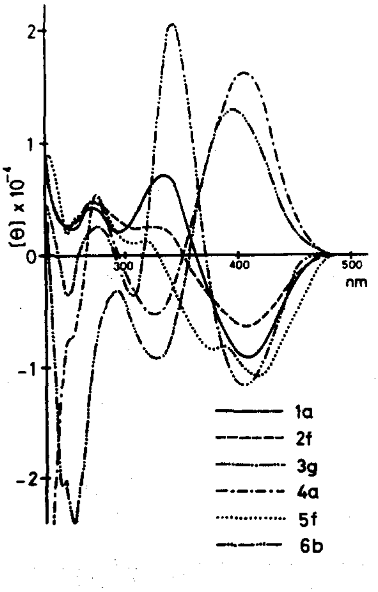


Fig. 2. CD spectra of some representative compounds.

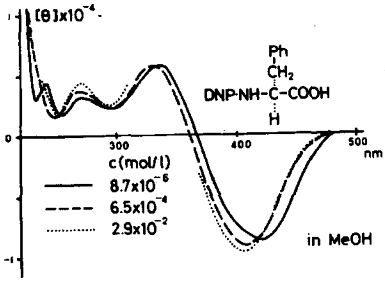


Fig. 3. Effect of concentration on the CD spectrum of DNP-L-phenylalanine.

In the early communication[1,3] CD spectra of the compounds **1** and **2** series were interpreted by exciton coupling of the transition moments of DNP and aromatic chromophores assuming the conformer having negative bond chirality between C_α-NH·DNP and C_β-Ar bonds to be predominant. But the assumption about the predominant conformer was shown to be incorrect by NMR study of **1a** in which one of the β-hydrogens was stereospecifically labelled with deuterium.[10] NMR spectra of all the DNP derivatives so far examined showed

Table 8. CD spectral data of *p*-methoxyanilides of DNP-L-α-amino acids with a non-aromatic side chain (general formula 7)

Compounds	R=	$\frac{\lambda(\mathrm{nm})}{[\theta]\times10^{-2}}$								
7a	$CH(CH_3)_2$	397	358	330	(290)	257	*245	233	227	214
		+149	0	-135	-50	-315	-270	0	+122	0
7b	$CH_2CH(CH_3)_2$	399	356	324	(289)	256	*245	233	223	(215)
		+137	0	-106	-48	-282	-235	0	+126	+37
7c	$CH(CH_3)CH_2CH_3$	400	359	328	(292)	255	*245	233	223	(213)
		+132	0	-128	-54	-286	-255	0	+110	+5
7d	CH_2OH	398	353	326	(291)	256	*245	232	222	211
		+126	0	-86	-48	-240	-220	0	+114	0
7e	$CH(OH)CH_3$	398	353	327	(293)	253	*245	231	224	216
		+153	0	-92	-53	-289	-268	0	+87	0
7f	CH_2CONH_2	393	356	324	(290)	252	233	233		
		+112	0	-108	-48	-232	0	+107		
7g	$CH_2CH_2CONH_2$	392	350	325	(290)	257	*245	236	228	(213)
		+75	0	-97	-36	-209	-162	0	+190	+107
7h	$CH_2CH_2SCH_3$	394	358	328	(290)	255	236	225		
		+88	0	-98	-34	-237	0	+194		

The upper row numbers for each compound indicate the wavelengths (nm) where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity values ($[\theta]\times10^{-2}$) at the wavelengths.

Table 9. CD spectra data of **1a** in various solvents

Solvents	$\frac{\lambda(\mathrm{nm})}{[\theta]\times10^{-2}}$								
EtOH	408	363	335	(296)	268	(250)			
	-96	0	+80	+26	+45	+26			
Me_2CO	407	360	338						
	-112	0	+74						
THF	404	361	332	(296)	268	(248)			
	-106	0	+67	+22	+46	+20			
Dioxane	402	358	333	(298)	262	245			
	-92	0	+46	+15	+41	0			
$CHCl_3$	402	355	335	(305)	265				
	-75	0	+28	+18	+56				
AcOH	401	358	330	(295)	264				
	-81	0	+50	+25	+45				
DMSO	421	373	342	(307)	280				
	-100	0	+70	+31	+45				
DMF	427	378	348	(310)					
	-103	0	+68	+12					
Pyridine	415	372	345						
	-85	0	+66						
4%$NaHCO_3$	430	381	345	(310)	283	264	*250	234	229
(aq.)	-56	0	+56	+25	+46	0	-26	-67	0

The upper row numbers for each compound indicate the wavelengths where maximal, zero, minimal (in parentheses), and shoulder (* marked) values of ellipticity were observed. The lower row numbers indicate molar ellipticity values at the wavelengths.

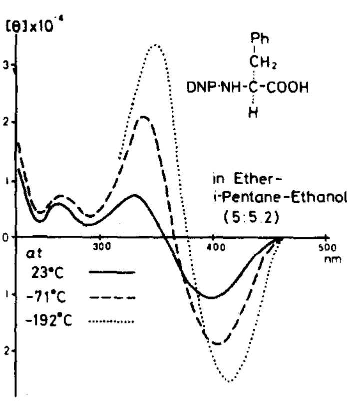


Fig. 4. Temperature dependence of the CD spectrum of DNP-L-phenylalanine.

similar values of the coupling constants (J^3_{NH-CH}=6 ~ 7 Hz), suggesting that DNP·NH group takes similar spatial arrangement around the chiral center in these compounds. Inspection of molecular model indicates that rotation about the DNP·NH–C_α bond is restricted due to the steric hindrance of the *o*-H atom if planar conformation is assumed for DNP·NH group by H-bonding between NH and *o*-NO_2 groups, and that only the conformers like A and B, in which α-H is nearly eclipsed with *o*-H atom seem possible for **8a**. Relative arrangements of the DNP and aromatic chromophores in the conformers A and B are similar in the sense that the aromatic group is located upside of the DNP·NH plane when projected as shown in Fig. 5. The arrangement is always the same for the compounds of general formula **8a**. In this arrangement the aromatic chromophore would give perturbation to the DNP·NH chromophore to result in characteristic pattern of CD spectrum. Probably this would be the reason for the applicability of the DNP-aromatic rule to a wide variety of compounds, though more elaborate study is necessary to elucidate the origin.

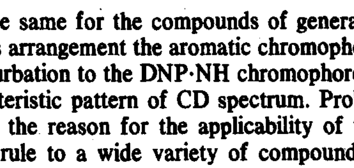


Fig. 5. Newman projection of two probable conformers of **8a**.

Recently, Bettoni *et al.*[9] have confirmed DNP-aromatic rule in the original form adding some other examples. They state also that the rule can be applied to aliphatic compounds. However, we think it dangerous because the sign of Cotton effect of such low intensity ($[\theta]^{max}_{400} < 2000$ in absolute value) may be readily reversed by some minor change in the conditions of CD measurements and in structure. Indeed, DNP-L-leucine show negative Cotton effect at $c = 3.2 \times 10^{-3}$ M, whereas positive sign at $c = 2.4 \times 10^{-4}$ in MeOH. DNP-L-methionine ($c = 1.9 \times 10^{-4}$ M) and DNP-L-arginine ($c = 1.1 \times 10^{-4}$ M) exhibit negative CD band near 400 nm in contrast to DNP derivatives of other aliphatic amino acid, which show positive Cotton effect at $c \simeq 10^{-4}$ M in MeOH.

Although many chromophoric derivatives of α-amino acids were reported to correlate their sign of Cotton effect to the absolute configuration, only a few among them were proved applicable generally to chiral amines other than α-amino acids.[11] Salicylideneimino derivatives were proved applicable to many chiral amine having an aromatic group, and Smith *et al.*[12] proposed salicylideneimino chirality rule to expalin their results. DNP-aromatic rule seems to have resemblance to the salicylideneimino chirality rule in the origin of CD spectra. The former, however, has now been proven applicable to much wider groups of compounds than the latter.

Compounds of **3**, **6** and **7** series as well as **4d** and **4e** are the first successful examples of deliberate introduction of two different chromophoric groups into a bifunctional aliphatic compound for determining their absolute configuration of CD spectra due to interaction of the two chromophores. The concept seems also promising for other bifunctional compounds having no primary amine if suitable derivatives could be devised.

In conclusion DNP-aromatic rule, which was originally found for DNP derivatives with an aromatic side chain, has been extended to the compounds of general formula **8** (i.e. $[\theta]^{max}_{400} < 0$ for **8a** and > 0 for **8b**). The rule provides a simple and useful method for determining absolute configuration of chiral amines in which primary amino group is attached directly to the asymmetric carbon. The method is applicable not only to the amines with an aromatic group in itself but also to those amines with a functional group into which an aromatic group can be introduced by suitable derivatization. Practical examples of such amines are amino acids, amino alcohols, amino thiols, diamines and so on. Although actual examples examined in this paper are confined to α-amino compounds because of availability, the rule should also be applicable to chiral amines in which the amino group is located at the other positions than α, e.g. β-amino acids, which occur sometimes as a component of peptide antibiotics.

EXPERIMENTAL

CD spectra were recorded on a JASCO spectropolarimeter model J-20 or J-40 in MeOH solution at room temp. except the spectra shown in Fig. 3, which were measured in ether-isopentane-EtOH (5:5:2) with a low temp. measurement attachment, and those in Table 9.

DNP-L-Phenylalanine (**1a**), N,O-*di-DNP-L-tyrosine* (**1c**), N^α, N^{im}-*di-DNP-L-histidine* (**1e**), and *DNP-L-tryptophane* (**1f**) were purchased from Wako Chemicals Ltd. and used for CD measurement without further purification.

Other compounds were prepared as described below. M.ps were uncorrected. Some of the DNP derivatives synthesized were oily or gummy mass or non-crystalline semisolid and contained solvent molecules which could not be removed completely even under vacuum for 3–4 days at the temp. higher than the b.p. of the solvent. Purity of these compounds was checked by SiO_2–TLC (benzene–EtOAc–EtOH, $CHCl_3$–MeOH–AcOH, or $CHCl_3$–acetone) and PMR spectra (Hitachi R-24 spectrometer; $CDCl_3$, acetone-d_6 or DMSO-d_6 solution) and CD spectra were corrected, if necessary, with respect to the solvent molecules included, which were shown by PMR spectra. Some of the non-crystalline derivatives were characterized by converting them into well crystalline dicyclohexylammonium (= DCHA) salts.

The following compounds were prepared by 2,4-dinitrophenylation of the commercially available compounds with the procedure of Sanger[13] (**1g**, **1j**, **3g**, **3k**, **5b**, Sigma; **1h**, **3b**, **3c**, **3d**, **3e**, **3f**, **5a**, Protein Research Foundation; **1i**, **6e**, **6i**, Bachem; **1k**, Aldrich; **3i**, Kokusan Chemicals).

N-*DNP*-O-*methyl-L-tyrosine* (**1g**). Yellow fine needles, m.p. 171–178° dec. (Found: C, 53.00; H, 4.20; N, 11.44. Calc. for $C_{16}H_{15}O_7N_3$: C, 53.19; H, 4.18; N, 11.63%).

N-*DNP*-O-*benzyl-L-tyrosine* (**1h**). Yellow powder from EtOAc, m.p. 160–165°. (Found: C, 60.44; H, 4.28; N, 9.70. Calc. for $C_{22}H_{19}O_7N_3$: C, 60.41; H, 4.38; N, 9.61%).

*DNP-L-*p-*nitrophenylalanine* (**1i**). Yellow prisms from $CHCl_3$, m.p. 174–176°. (Found: C, 47.23; H, 3.23; N, 14.38. Calc. for $C_{15}H_{12}O_8N_4 \cdot 1/4H_2O$: C, 47.31; H, 3.31; N, 14.71%).

N,O$^{3'}$,O$^{4'}$-*Tri-DNP-3',4'-dioxy-L-phenylalanine* (**1j**). Yellow powder from EtOAc-ether-hexane, m.p. 108–115°. (Found: C, 46.29; H, 2.47; N, 13.85. Calc. for $C_{27}H_{17}O_{16}N_7$: C, 46.63; H, 2.46; N, 14.10%).

N,O-*Di-DNP-mimosine* (**1k**). Yellow powder from acetone-EtOAc, m.p. 165–167°. (Found: C, 44.23; H, 2.66; N, 15.32. Calc. for $C_{20}H_{14}O_{12}N_6 \cdot 1/2H_2O$: C, 44.54; H, 2.80; N, 15.58%).

N-*DNP*-O-*benzyl-L-serine* (**3b**). Yellow viscous oil.

N-*DNP*-S-*benzyl-L-cysteine* (**3c**). Yellow glassy mass.

DNP-*L-aspartyl β-benzyl ester* (**3d**). Yellow oil.

DNP-*L-aspartyl β-*p-*nitroanilide* (**3e**). Yellow needles from EtOH-H_2O, m.p. 190–192° dec. (Found: C, 45.58, H, 3.12; N, 16.83. Calc. for $C_{16}H_{13}O_9N_5$: C, 45.83; H, 3.13; N, 16.70%).

DNP-*L-glutamyl γ-benzyl ester* (**3f**). Yellow oil which crystallized on standing, m.p. 125–127°. (Found: C, 53.59; H, 4.23; N, 10.52. Calc. for $C_{18}H_{17}O_8N_3$: C, 53.60; H, 4.25; N, 10.42%).

DNP-*L-glutamyl γ-*p-*nitroanilide* (**3g**). Yellow powder precipitated by acidification of the aqueous alkaline solution m.p. 210–215° dec. (Found: C, 47.16; H, 3.73; N, 16.24. Calc. for $C_{17}H_{15}O_9N_5$: C, 47.12, H, 3.49, N, 16.16%).

N$^{\alpha}$-*DNP*-N$^{\delta}$-*carbobenzoxy-L-ornithine* (**3i**). Yellow viscous oil.

N$^{\alpha}$-*DNP*-N$^{\epsilon}$-*carbobenzoxy-L-lysine* (**3k**). Yellow viscous oil.

DNP-L-phenylalanyl ethyl ester (**5a**). Yellow prisms from benzene, m.p. 105–107°. (Found: C, 56.73; H, 4.45; N, 11.84. Calc. for $C_{17}H_{17}O_6N_3$: C, 56.82; H, 4.77; N, 11.70%).

DNP-L-phenylalanyl amide (**5d**). Yellow powder from EtOAc, m.p. 178–180°. (Found: C, 54.05; H, 4.38; N, 16.52. Calc. for $C_{15}H_{14}O_5N_4 \cdot 1/4H_2O$: C, 53.81; H, 4.37; N, 16.73%).

DNP-L-alanyl β-naphthylamide (**6e**). Yellow prisms from acetone-EtOAc, m.p. 218–219°. (Found: C, 59.90; H, 4.12; N, 14.61. Calc. for $C_{19}H_{16}O_5N_4$: C, 60.00, H, 4.24; N, 14.73%).

DNP-L-alanyl benzyl ester (**6i**). Yellow prisms from benzene, m.p. 112–113°. (Found: C, 55.94; H, 4.57; N, 12.13. Calc. for $C_{16}H_{15}O_6N_3$: C, 55.65; H, 4.38; N, 12.17%).

The DNP derivatives, **1l**, **2a**–**2e**, and **2f**, were also prepared by 2,4-dinitrophenylation[13] of the samples, supplied from Dr. S. Inouye at Meiji Seika Ltd., Professor R. Nakashima at Tottori University, and Professor N. Izumiya at Kyusyu University, respectively.

N,O-*Di-DNP-β-(5-hydroxy-2-pyridyl)-L-alanine* (**1l**). Yellow powder from EtOAc-ether, m.p. 112–118° dec.

DNP-S-phenylglycine (**2a**). Yellow semisolid. DCHA salt: Yellow needles from EtOAc-hexane, m.p. 183–184°. (Found: C, 62.94; H, 6.91; N, 11.22. Calc. for $C_{14}H_{11}O_6N_3 \cdot C_{12}H_{23}N$: C, 62.63; H, 6.87; N, 11.24%).

DNP-S-(2-phenylethyl)glycine (**2b**). Yellow oil. DCHA salt: Yellow powder from ether-hexane, m.p. 164–166°. (Found: C, 63.33; H, 7.03; N, 10.56. Calc. for $C_{16}H_{15}O_6N_3 \cdot C_{12}H_{23}N \cdot 1/4H_2O$: C, 63.32; H, 7.31; N, 10.55%).

DNP-S-(3-phenylpropyl)glycine (**2c**). Yellow semisolid. DCHA salt: Yellow needles from EtOAc-hexane, m.p. 174–178°. (Found: C, 64.16; H, 7.50; N, 10.12. Calc. for $C_{17}H_{17}O_6N_3 \cdot C_{12}H_{23}N$: C, 64.42; H, 7.46; N, 10.36%).

DNP-S-(4-phenylbutyl)glycine (**2d**). Yellow oil. DCHA salt: Yellow powder from benzene-hexane, m.p. 162–163°. (Found: C, 64.80; H, 7.57; N, 9.98. Calc. for $C_{18}H_{19}O_6N_3 \cdot C_{12}H_{23}N$: C, 64.96; H, 7.63; N, 10.10%).

DNP-S-(5-phenylpentyl)glycine (**2e**). Yellow oil. DCHA salt: Yellow prisms from EtOAc-hexane, m.p. 128–130°. (Found: C, 64.98; H, 7.59; N, 9.70. Calc. for $C_{19}H_{21}O_6N_3 \cdot C_{12}H_{23}N \cdot 1/4H_2O$: C, 64.96; H, 7.82; N, 9.77%).

*DNP-S-(3-*p-*methoxyphenylpropyl)glycine* (**2f**). Yellow oil which solidified on standing. m.p. 113–115°. (Found: C, 55.41; H, 4.95; N, 10.40. Calc. for $C_{18}H_{19}O_7N_3$: C, 55.52; H, 4.92; N, 10.79%).

DNP-L-alanyl anilide (**6a**). Aniline (47 mg, 0.5 mmol) and DNP-L-alanyl N-hydroxysuccinimide ester (88 mg, 0.25 mmole; m.p. 148–152°), prepared from DNP-L-alanine as described in literature[14] in DMF (1 ml) were stirred overnight. To the reaction mixture was added 5% $NaHCO_3$ aq and yellow precipitate was collected, washed with water, and recrystallized from benzen-acetone. Yellow powder, m.p. 181–183°. (Found: C, 54.79; H, 4.29; N, 16.83. Calc. for $C_{15}H_{14}O_5N_4$: C, 54.54; H, 4.27; N, 16.96%).

The following were prepared in the similar manner as **6a**.

DNP-L-alanyl p-*methoxyanilide* (**6b**). Yellow fine needles from EtOAc, m.p. 207–211°. (Found: C, 53.44; H, 4.28; N, 15.56. Calc. for $C_{16}H_{16}O_6N_4$: C, 53.33; H, 4.48; N, 15.55%).

DNP-L-alanyl p-*dimethylaminoanilide* (**6c**). This compound was obtained as dark brown powder though its solution was yellow and gave normal absorption spectrum [$\lambda_{\max}^{\mathrm{MeOH}}$ 400 sh (ϵ 6000), 342 (18100), and 268 nm (21700)].

DNP-L-alanyl p-*nitroanilide* (**6d**). N-(t-Butoxycarbonyl)-L-alanine (568 mg, 3 mmol), *p*-nitroaniline (690 mg, 5 mmol), and dicyclohexylcarbodiimide (722 mg, 3.5 mmol) in THF (10 ml) were stirred for 2 hr at −10° and for 24 hr at room temp. After usual work-up the solid residue was washed with hot water to remove *p*-nitroaniline and recrystallized from ether to give N-(t-butoxycarbonyl)-L-alanyl *p*-nitroanilide as colorless needles (510 mg, 55%): m.p. 130–135°. This compound (103 mg, 0.33 mmol) was treated with trifluoroacetic acid (1 ml) to give L-alanyl-*p*-nitroanilide trifluoroacetate, which was dinitrophenylated without purification to give **6d** (75 mg, 60%). Recrystallization from EtOAc gave yellow needles: m.p. 222–228°. (Found: C, 47.66; H, 3.58; N, 18.27. Calc. for $C_{15}H_{13}O_7N_5$: C, 48.00; H, 3.49; N, 18.66%).

DNP-L-alanyl benzylamide (**6f**). Yellow fine needles from EtOAc, m.p. 160–162°. (Found: C, 55.72; H, 4.66; N, 16.18. Calc. for $C_{16}H_{16}O_5N_4$: C, 55.81; H, 4.68; N, 16.27%).

DNP-L-alanyl β-phenethylamide (**6g**). Yellow needles from benzene, m.p. 115–117°. (Found: C, 56.72; H, 5.03; N, 15.67. Calc. for $C_{17}H_{18}O_5N_4$: C, 56.98; H, 5.06; N, 15.64%).

DNP-L-alanyl phenyl ester (**6h**). Yellow oil.

DNP-L-alanyl β-phenethyl ester (**6j**). Yellow oil.

DNP-L-alanyl γ-phenylpropyl ester (**6k**). Yellow oil.

DNP-L-valyl p-*methoxyanilide* (**7a**). To a solution of DNP-L-valine (42 mg, 0.15 mmole) and *p*-anisidine (25 mg, 0.2 mmol) in DMF (1 ml) at −10° were added dicyclohexylcarbodiimide (42 mg, 0.2 mmol) and 1-hydroxybenzotriazole (20 mg, 0.15 mmol). After stirring the mixture overnight at 5°, aqueous soln of citric acid was added and the ppt was collected and dissolved in EtOAc. Usual work-up and crystallization from EtOAc gave **7a** as yellow needles, m.p. 173–174°. (Found: C, 55.43; H, 5.18; N, 14.34. Calc. for $C_{18}H_{20}O_6N_4$: C, 55.67; H, 5.19; N, 14.43%).

The following were also synthesized from DNP-L-amino acids and amino compounds similarly as **7a**.

DNP-L-phenylalanyl dimethylamide (**5c**). Yellow oil which solidified on standing, m.p. 112–115°. (Found: C, 55.79; H, 5.07; N, 15.32. Calc. for $C_{17}H_{18}O_5N_4 \cdot 1/2H_2O$: C, 55.58; H, 5.21; N, 15.25%).

DNP-L-leucyl p-*methoxyanilide* (**7b**). Yellow powder from EtOH-H_2O, m.p. 214–225° dec. (Found: C, 56.71; H, 5.62; N, 13.60. Calc. for $C_{19}H_{22}O_6N_4$: C, 56.71; H, 5.51; N, 13.92%).

DNP-L-isoleucyl p-*methoxyanilide* (**7c**). Yellow powder from EtOAc–hexane, m.p. 175–177°. (Found: C, 56.53; H, 5.48; N, 13.68. Calc. for $C_{19}H_{22}O_6N_4$: C, 56.71; H, 5.51; N, 13.92%).

DNP-L-seryl p-*methoxyanilide* (**7d**). Yellow powder from MeOH-Et_2O, m.p. 147–150° dec. (Found: C, 50.97; H, 4.45; N, 15.15. Calc. for $C_{16}H_{16}O_7N_4$: C, 51.06; H, 4.29; N, 14.89%).

DNP-L-threonyl p-*methoxyanilide* (**7e**). Yellow powder from acetone-hexane, m.p. 162–163°. (Found: C, 51.75; H, 4.78; N, 14.17. Calc. for $C_{17}H_{18}O_7N_4 \cdot 1/4H_2O$: C, 51.71; H, 4.72; N, 14.19%).

DNP-L-asparaginyl p-*methoxyanilide* (**7f**). Yellow powder from acetone-EtOAc, m.p. 240–243° dec. (Found: C, 50.63; H, 4.18; N, 17.08. Calc. for $C_{17}H_{17}O_7N_5$: C, 50.62; H, 4.25; N, 17.36%).

DNP-L-glutaminyl p-*methoxyanilide* (**7g**). Yellow powder from acetone-hexane, m.p. 203–204°. (Found: C, 51.55; H, 4.47; N, 16.51. Calc. for $C_{18}H_{19}O_7N_5$: C, 51.80; H, 4.59; N, 16.78%).

DNP-L-methionyl p-methoxyanilide (**7h**). Yellow fine needles from $CHCl_3$-benzene, m.p. 138–139°. (Found: C, 51.43; H, 4.79; N, 13.26. Calc. for $C_{18}H_{20}O_6N_4S$: C, 51.42; H, 4.79; N, 13.33%).

N-DNP-L-tyrosine (**1b**). A soln of 500 mg (1.14 mmol) of benzyl ether (**1h**) in acetone (30 ml) and 30% HBr aq. (33 ml) was refluxed for 30 hr. The debenzylated product (**1b**) was purified by column chromatography (SiO_2; benzene–EtOAc) and obtained as yellow viscous oil.

N^{a}-*DNP-L-histidine* (**1d**). L-Histidine·HCl·H_2O (210 mg, 1 mmol), 1-chloro-2,4-dinitrobenzene (203 mg, 1 mmol), and $NaHCO_3$ (336 mg, 4 mmol) in EtOH (2–1 ml) and H_2O (0.34 ml) were stirred for 72 hr at room temp. Crystallization from H_2O gave 37 mg of **1d**, m.p. 250–280° dec. This compound is assumed to be in zwitterionic form because of the absence of 1R band assignable to CO_2H. (Found: C, 44.74; H, 3.40; N, 22.00. Calc. for $C_{12}H_{11}O_6N_5$: C, 44. 86; H, 3.45; N, 21.80%).

N-*DNP-O-benzoyl-L-serine* (**3a**). DNP-L-serine (270 mg, 1 mmol) was benzoylated with Schotten-Baumann method using benzoyl chloride (2 mmol) and NaOH (3.5 mmol) at 0–5°. Column chromatography (SiO_2; benzene-EtOAc-MeOH) gave 135 mg of **3a**, m.p. 181–184° from EtOAc. (Found: C, 50.95; H, 3.51; N, 10.97. Calc. for $C_{16}H_{13}O_8N_3$: C, 51.20; H, 3.49; N, 11.20%).

N-*DNP-O-benzoyl-L-homoserine* (**3h**). DNP-L-homoserine (m.p. 137–138°, prepared in the usual way) in MeOH was treated with excess CH_2N_2 in ether, and the resulting methyl ester (yellow oil) was benzoylated with benzoyl chloride in pyridine to give methyl ester of **3h** as yellow oil. The methyl ester (220 mg, 0.55 mmol) in acetone was subjected to hydrolysis by addition of 0.5N–NaOH (1.3 ml). Usual work-up and column chromatography (SiO_2; benzene-EtOAc) gave **3h** (100 mg), as yellow semisolid. Benzoylation of DNP-L-homoserine did not afford 3 h but DNP-L-homoserine lactone, M.P. 143–145°.

N^{α}-*DNP*-N^{δ}-*benzoyl-L-ornithine* (**3j**). The derivative **3i** was stirred in 25% HBr–AcOH for 30 min at room temp. After evaporation of HBr and AcOH the residue was subjected to Schotten-Baumann benzoylation. Column chromatography (SiO_2; benzene-EtOAc) gave **3j** as yellow oil.

N^{α}-*DNP*-N^{ϵ}-*benzoyl-L-lysine* (**3l**). This compound was prepared from **3k** in the similar manner as above and obtained as a yellow oil.

N-*DNP-S-α-phenylethylamine* (**4a**). Yellow viscous oil. (Found: C, 58.41; H, 4.65; N, 14.34. Calc. for $C_{14}H_{13}O_4N_3$: C, 58.53; H, 4.56; N, 14.63%).

N-*DNP*-3S-*amino*-1-*phenylbutane* (**4b**). 3S-Acetamido-1-phenylbutane† (96 mg, 0.5 mmol; $[\alpha]_D^{23}+38.9°$, *c* 0.7 EtOH, lit.,[8] enantiomer $[\alpha]_d^{26}-35.2°$, *c* (0.7 EtOH) was dissolved in conc. HCl–AcOH (1:1) and maintained at 110° for 24 hr in a sealed tube. The deacetylated compound was treated with 1-fluoro-2.4-dinitrobenzene and $NaHCO_3$ in aqueous acetone to give **4b**. Column chromatography (SiO_2; benzene-hexane) and crystallization from benzene-hexane afforded yellow prisms (140 mg), m.p. 50–51°. (Found: C, 61.20; H, 5.34; N, 13.38. Calc. for $C_{16}H_{17}O_4N_3$: C, 60.94; H, 5.43; N, 13.33%).

N-*DNP*-3S-*amino*-1-*phenylbut*-1-*ene* (**4c**). 3S-Amino-1-phenylbut-1-ene L-tartrate† (90 mg, 0.3 mmol; $[\alpha]_D^{21}-3.2°$, *c* 0.2 EtOH) was treated with 1-fluoro-2.4-dinitrobenzene and $NaHCO_3$ in aqueous acetone to give **4c**. Preparative TLC (SiO_2; benzene) followed by crystallization from CCl_4 afforded yellow prisms (65 mg), m.p. 108–109°. (Found: C, 61.23; H, 4.80; N, 13.50. Calc. for $C_{16}H_{15}O_4N_3$: C, 61.33; H, 4.83; N, 13.41%).

N-*DNP-O-benzoyl-L-alaninol* (**4d**). DNP-L-alaninol (330 mg, 1.37 mmol; m.p. 94–95°, prepared from L-alaninol as usual) was benzoylated with benzoyl chroride (2.05 mmol) in pyridine (2.2 ml) affording **4d**, yellow needles from benzene-hexane, m.p. 89–92°. (Found: C, 55.73; H, 4.38; N, 11.99. Calc. for $C_{16}H_{15}O_6N_3$: C, 55.65; H, 4.38; N, 12.17%).

N^2-*DNP*-N^1*benzoyl*-R-1,2-*diaminopropane* (**4e**).‡ To a stirred soln of *R*-1,2-diaminoporpane bis-L-tartrate (0.75 g, 2 mmol; m.p. 142–143°, $[\alpha]_D^{23}+21.2°$, *c* 1.63 H_2O) and $NaHCO_3$ (1.5 g) in H_2O (20 ml) was added benzoyl chloride (2.1 mmol). After stirring for 2 hr 1-fluoro-2.4-dinitrobenzene (4 mmol) in acetone (20 ml) was added and stirred overnight. The crude product was subjected to preparative TLC (SiO_2; developed 7 times with $CHCl_3$) and yellow main band was collected to afford **4e** as yellow semisolid.

DNP-L-Phenylalanylglycine (**5d**). DNP-L-phenylalanylglycyl methyl ester (m.p. 143–145°; 110 mg, 0.27 mmol), synthesized from **1a** and glycyl methyl ester hydrochloride using dicyclohexylcarbodiimide, was stirred in a mixture of NaOH (1 ml) and MeOH (8 ml) at room temperature for 1 hr. Usual work-up followed by column chromatography (SiO_2; $CHCl_3$–MeOH) gave **5d** (65 mg). Yellow powder from benzene-EtOAc, m.p. 79–84°. (Found: C, 53.96; H, 4.54; N, 13.82. Calc. for $C_{17}H_{16}O_7N_4 \cdot 1/6C_6H_6$: C, 53.86; H, 4.27; N, 13.96%).

N-(*DNP-L-phenylalanyl*)-*N,N'*;-*dicyclohexylurea* (**5e**). This compound was obtained as a byproduct in the synthesis of **5d**. Yellow powder, m.p. 76–80°. (Found: C, 62.47; H, 6.69; N, 12.72. Calc. for $C_{28}H_{35}O_6N_5$: C, 62.55; H, 6.56; N, 13.03%).

DNP-L-phenylalaninol (**5f**). L-Phenylalaninol was synthesized from L-phenylalanine as described in literature[15] and was 2.4-dinitrophenylated as usual. Yellow powder from MeOH–H_2O, m.p. 57–63°.

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†These samples were supplied from Prof. Y. Inouye at Kyoto University.

‡[θ] values given in Table 4 are those of enantiomer of **4e**.