Table I. Preparation of 3a from 1, Acetamide, Paraformaldehyde, and Concentrated H_2SO_4

expt	mol of CH_3CONH_2	reaction conditions		% yield[a]	
		temp, °C	time, h	3a	5
1	1.0	23	24	0	
2	1.0	55	3	26	25
3	1.0	55	24	26	
4	1.0	90	7	0	50
5	2.0	55	10	62	8
6	3.0	23	24	30	1
7	3.0	55	8	75	4

[a] Determined by NMR of crude product.

Table II. Preparation of 4 from 3a and $POCl_3$

expt	molar ratio of $POCl_3$/3a	reaction conditions			% yield of 4
		solvent	time, h	temp, °C	
1	2.1[a]	xylene	1	144	85
2	2.1	chlorobenzene	1.5	135	67
3	1.1	chlorobenzene	5	135	30
4	1.5	*N,N*-dimethylformamide	3	125	58
5	3	$POCl_3$	3	105	50
6	1.1[b]	toluene	16	110	40
7	1.1	ethylene dichloride	6	84	50

[a] DMF (1.0 mol) was used. [b] DMF (1.3 mol) was used.

at 90 °C and filtered. Cooling the solution gave 3.9 g (38%) of **3a**. The insoluble material (2.5 g) was crude diarylmethane **5**.

With 8.90 g (0.10 mol) of *N*-(hydroxymethyl)acetamide, a 65% yield of **3a** was isolated after 8 h at 55 °C.

***N*-(2-Methyl-5-nitrobenzyl)benzamide (3b).** To a solution of 6.85 g (0.05 mol) of *p*-nitrotoluene in 60 mL of concentrated H_2SO_4 was added 7.55 g (0.05 mol) of *N*-(hydroxymethyl)benzamide.[7] The solution was stirred at room temperature for 63 h and poured into ice and water. Recrystallization three times from ethanol gave 4.0 g (30%) of colorless **3b**: mp 134–137 °C; IR, 3300, 1635, 1655 (sh) cm^{-1}; mass spectrum, *m/e* 270 (M^+). Anal. Calcd for $C_{15}H_{14}N_2O_3$: C, 66.67; H, 5.78; N, 10.37. Found: C, 66.54; H, 5.10; N, 10.12.

A reaction time of 7 days gave **3b** in 43% yield.

(7) Monti, L. *Gazz. Chem.* **1930**, *50*, 39; *Chem. Abstr.* **1930**, *24*, 4013.

2-(Chloromethyl)-4-nitrotoluene (4). (a) From 3a. A solution of 6.24 g (0.03 mol) of **3a**, 9.67 g (0.063 mol) of $POCl_3$, and 2.19 g (0.03 mol) of DMF in 50 mL of xylene was refluxed 1 h. The cooled solution was washed with water and evaporated to give 4.73 g (85%) of **4**, mp 61–62 °C (after recrystallization from hexane) (lit.[1b] mp 63–64 °C).

Caution: **4** is a lachrymator and may be a skin irritant.

(b) From 3b. A solution of 2.70 g (0.01 mol) of **3b** and 6.12 g (0.04 mol) of $POCl_3$ was refluxed for 3 h. The cooled solution was stirred in water for 30 min, and the resulting two layers were extracted with chloroform. Evaporation gave 1.5–2 g of oil; the IR showed a mixture of **4** and benzonitrile (2225 cm^{-1}).

Registry No. 1, 99-99-0; **2a**, 60-35-5; **3a**, 86392-53-2; **3b**, 86392-54-3; **4**, 58966-24-8; **5**, 86409-50-9; **6a**, 625-51-4; *N*-(hydroxymethyl)benzamide, 6282-02-6.

Communications

Mixed Anhydrides in Peptide Synthesis. Reduction of Urethane Formation and Racemization Using *N*-Methylpiperidine as the Tertiary Amine Base

Summary: The side reactions of urethane formation and racemization accompanying couplings by the mixed anhydride method are reduced when *N*-methylpiperidine is used as base, the best results being achieved in dichloromethane.

Sir: One of the popular methods of coupling in peptide synthesis involves activation of the *N*-(alkoxycarbonyl)-amino or protected peptide acid by formation of the anhydride with a carbonic acid monoester.[1,2] This mixed carboxylic acid–carbonic acid anhydride **3** is generated by reaction of the acid **1** with an alkyl chloroformate (**2**) in the presence of a tertiary amine base. Aminolysis by the nucleophile (**4**) produces the peptide **5**. The method is quick and efficient for chain buildup by the successive addition of single residues.[2,3] Conditions for minimizing racemization during the coupling of peptide acids have been defined.[4,5] Unfortunately, a second acylation product (**6**), namely, a urethane formed by attack of the nucleophile at the carbonic acid carbonyl, results from the aminolysis of the mixed anhydride (see ref 2 and 3). More urethane is produced when the activated residue is valyl or isoleucyl,[6] but little else is known about the reaction. This

(1) Boissonnas, R. A. *Helv. Chim. Acta* **1951**, *34*, 874. Vaughan, J. R. *J. Am. Chem. Soc.* **1951**, *73*, 3547. Vaughan, J. R.; Osata, R. L. *Ibid.* **1952**, *74*, 676. Wieland, T.; Bernhard, H. *Liebigs Ann. Chem.* **1951**, *572*, 190. For reviews, see: Albertson, N. F. *Org. React. (N.Y.)* **1962**, *12*, 157. Tarbell, D. S. *Acc. Chem. Res.* **1969**, *2*, 296 and ref 2.

(2) Meienhofer, J. In "The Peptides, Analysis, Synthesis, Biology"; Gross, E., Meienhofer, J., Eds.; Academic Press: New York, 1979; Vol. 1, p 263.

(3) Beyerman, H. C.; de Leer, E. W. B.; Floor, J. *Recl. Trav. Chim. Pays-Bas* **1973**, *92*, 481.

(4) Anderson, G. W.; Zimmerman, J. E.; Callahan, F. M. *J. Am. Chem. Soc.* **1967**, *89*, 5012. Anderson, G. W. In "Peptides: Chemistry and Biology"; Weinstein, B., Lande, S., Eds.; Marcel Dekker: New York, 1970; p 255.

(5) Wieland, T.; Faesel, J.; Konz, W. *Liebigs Ann. Chem.* **1969**, *722*, 197. Wieland, T.; Faulstich, H.; Fahrenholz, F. *Ibid.* **1971**, *743*, 77.

(6) Bodanszky, M.; Tolle, J. C. *Int. J. Pept. Protein Res.* **1977**, *10*, 380.

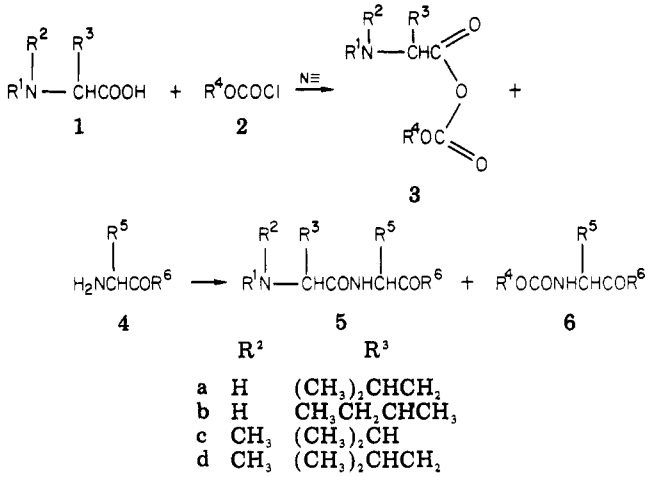
0022-3263/83/1948-2939$01.50/0

Table I. Yields (%) of Protected Peptide 5 and Side Product 6a from Reactions of Acids 1 and Ester 4a by the Mixed Anhydride Method Using Isobutyl Chloroformate 2a and Different Tertiary Amines[a]

		triethylamine			*N*-methylmorpholine			*N*-methylpiperidine		
1	solvent	5	6a	ratio[b]	5	6a	ratio	5	6a	ratio
Boc-Leu (1a)	THF	89	0.25	(0.28)	91	0.24	(0.26)	90	0.22	(0.24)
	THF					1.48[c]				
Boc-Ile (1b)	DMF	86.2	6.5	(7.0)	87.3	5.1	(5.5)	93.9	5.5	(5.5)
	THF	53.5	33.7	(38.6)	82.1	5.0	(5.7)	83.0	4.3	(4.9)
	THF					6.5[c]				
	DCM	32.5	60.9	(65.2)	90.3	3.2	(3.4)	87.0	2.5	(2.8)
Boc-MeVal (1c)	DMF	65	21	(24)	55.2	17.0	(23.5)	63.7	22.0	(25.7)
	THF	30	60	(67)	64.8	20.8	(24.3)	74.2	22.3	(23.1)
	DCM	0	>90	(>90)	50[d]	11	(14)[d]	84.6	9.9	(10.5)
Boc-MeLeu (1d)	THF					1.8			1.0	
Z-MeVal[e]	THF				46	30	(39.5)	70	20	(22)
	DCM							72	8	(10)

[a] 2a (1.0 mmol) was added to 1 (1.05 mmol) and amine (1.0 mmol) in 20 mL of solvent at -5 °C. After 90 s, a cold solution of 4·HCl (1.0 mmol) and amine (1.0 mmol) was added. After 1 h at -5 °C and >3 h at 23 °C, the neutral products were collected after being washed with aqueous citric acid and $NaHCO_3$. [b] ratio = (100 × yield of 6a)/(yield of 6a + 5). [c] No excess of 1 was used. [d] 18% of *N*-carboxyanhydride formed. This amount was added to 50 for calculating the ratio. [e] Z = $C_6H_5CH_2OCO$; ratio determined by NMR.

Scheme I[a]



[a] a-d: $R^1 = (CH_3)_3COCO$; $R^4 = (CH_3)_2CHCH_2$; $R^5 = C_6H_5CH_2OCONH(CH_2)_4$; $R^6 = CH_3O$.

single unattractive feature of the method has tended to restrict the use of mixed anhydrides in peptide synthesis.

We have investigated this side reaction by comparing the yields of 5 and 6 obtained from reactions of 1 (R^1 = ROCO, ROCONHCHR(CO) with 4 (R^6 = OR) under various conditions, first using ^1H NMR for monitoring and then high-performance liquid chromatography.[7] Typical results appear in Table I. From a study involving more than 100 couplings, we report the following conclusions.

The extent of urethane formation is primarily dictated by the amine–solvent combination. In THF, the traditionally used solvent[4,5] Et_3N gives rise to much more urethane than *N*-methylmorpholine (NMM);[8] but in DMF,[3] the difference is marginal. In dichloromethane (DCM), two extremes obtain: NMM/DCM produces the least urethane, Et_3N/DCM, the most, so much so that it predominates for hindered residues. Use of 5% excess of acid 1[3,6] diminishes the amount of urethane. On the basis of our findings and previous information,[2,4] we searched for a better base and found that *N*-methylpiperidine (NMP)[9] is superior to NMM, particularly in apolar solvents (Table I), because it gives higher yields of desired peptide in most cases, and less urethane is formed.[10] In addition, NMP leads to less racemization during coupling of protected peptide acids than NMM (Table II).[11] We recommend NMP for routine use in the mixed anhydride method of coupling and DCM as the best solvent so far identified for minimizing urethane formation. Our conclusion on DCM contradicts the generally held notion that halogen-containing solvents are not good solvents for mixed anhydride couplings.[2] A second notion with which we disagree is that strictly anhydrous solvents are essential for mixed anhydride formation.[2] We have found that couplings[12] carried out totally in DMF or in DMF–H_2O (4:1)[13] gave the same good yields with no difference in the amount of urethane produced.

Table II. Racemization (%) during Coupling of Protected Dipeptide Acids with Ester 4a ($R^6 = C_6H_5CH_2O$)[a]

	Z-Gly-Val		Z-Gly-Phe	
amine	DCM	DMF	DCM	DMF
i, NMM	0.54	57	2.70	9.4
ii, NMP	0.46	32	1.76	7.8
% decrease[b]	15	44	35	17

[a] Reactions carried out as in Table I.[11] % Racemization = (100 × 2 × % D-L)/(% L-L + % D-L). [b] 100(i − ii)/i.

(7) Products were separated by HPLC, and yields were determined by measuring absorbance at 215 nm. Conditions: Waters 6000A (U6K injector, 450 detector) equipped with a 730 data processor and 10-μm μBondapak-C_{18} column, 30 cm × 3.9 mm i.d.; solvent, CH_3CN–H_2O (1:1) at 1.0 mL/min. Retention times and relative absorbances: **6a**, 13.4 min, 100.0; **5a**, 18.91 min, 103.0; **5b**, 17.3 min, 101.1; **5c**, 22.00 min, 113.7. Reference compound **6a** (oil) was prepared by reaction of **2a** with **4a** in the presence of excess NMM. Reference peptides **5a–d** were prepared as in Table I using NMM in DCM. After workup, products were recrystallized twice.

(8) NMM is the favored amine base in peptide synthesis because less racemization accompanies its use. It can also be inferred from the literature that higher yields are obtained by the mixed anhydride method using NMM rather than Et_3N. That urethane formation is central to this difference has never been demonstrated.

(9) Both NMP and NMM were examined in the classical work,[4] but NMM was selected for further study.

(10) In not one case did we find NMP to be inferior to NMM.

(11) Deprotected epimeric peptides were determined by HPLC (UV, 208 nm)[7] by using 0.01 M NH_4OAc as solvent. Gly-Val-Lys: $k'_{(L-L)}$ 1.08, $k'_{(D-L)}$ 2.17, *R* 4.08. Gly-Phe-Lys: $k'_{(L-L)}$ 6.38, $k'_{(D-L)}$ 12.25, *R* 4.98.

(12) 1, R^1 = ROCO or ROCONHCHRCO; 2, R^4 = Et; 4. Product ratio determined by NMR.

(13) Solvent used successfully for coupling symmetrical anhydrides of *N*-(alkoxycarbonyl)amino acids. Benoiton, N. L.; Chen, F. M. F. *FEBS Lett.* **1981**, *125*, 104.

Acknowledgment. This work was supported by a grant from the Medical Research Council of Canada. N.L.B. is a Career Investigator of the MRC.

Registry No. **1a**, 13139-15-6; **1b**, 13139-16-7; **1c**, 45170-31-8; **1d**, 53363-89-6; **2a**, 543-27-1; **5a**, 37571-12-3; **5b**, 23010-53-9; **5c**, 86632-69-1; **5c** (R^1 = $PhCH_2OCl(O)$), 86632-70-4; **6a**, 86645-64-9; H-Lys(Z)-OMe, 24498-31-5; H-Lys(Z)-OCH_2Ph, 24458-14-8; Z-MeVal, 42417-65-2; Z-Gly-Val, 33912-87-7; Z-Gly-Phe, 1170-76-9; Z-Gly-Val-Lys(Z)-OCH_2Ph, 86632-71-5; Z-Gly-Phe-Lys(Z)-OCH_2Ph, 86632-72-6; triethylamine, 121-44-8; *N*-methylmorpholine, 109-02-4; *N*-methylpiperidine, 626-67-5.

Francis M. F. Chen, Rene Steinauer
N. Leo Benoiton*

Department of Biochemistry
University of Ottawa Health Sciences
451 Smyth Road, Ottawa, Ontario K1H 8M5

Received April 11, 1983

Formation of a Novel Type of Adduct between an NADH Model and Carbonyl Compounds by Photosensitization Using $Ru(bpy)_3^{2+}$ [1]

Summary: The $Ru(bpy)_3^{2+}$-photosensitized reaction of 1-benzyl-1,4-dihydronicotinamide with several aromatic carbonyl compounds gave 1:1 adducts of the 4-substituted dihydronicotinamide structure in moderate to good yields along with less efficient reduction or no reduction to the corresponding alcohols; di-2-pyridyl ketone was exclusively reduced to the alcohol.

Sir: Reactions of 1,4-dihydropyridines have received much attention in relation with biological processes involving NAD(P)H.[2] Although extensive studies are focused on reductions of unsaturated substrates from mechanistic and synthetic points of view, addition of nucleophiles to the C_5–C_6 double bond of NADH models has also been well documented.[3] Reactions of NADH models with aromatic carbonyl compounds in aqueous solution lead to addition of both water and substrate molecules to the C_5–C_6 bond, giving 1:1:1 adducts.[4-6] The adduct formation was suggested to be of mechanistic importance in relation with reduction of carbonyl compounds.[5,6] We report here that the photosensitized reactions of 1-benzyl-1,4-dihydronicotinamide (BNAH) with aromatic carbonyl compounds **1a–f** by $Ru(bpy)_3^{2+}$ (bpy = 2,2′-bipyridine) afford a novel type of 1:1 adducts **3a–e** unlike the products of dark reactions. This type of adduct formation has only a few precedents in reactions with olefins.[7,8]

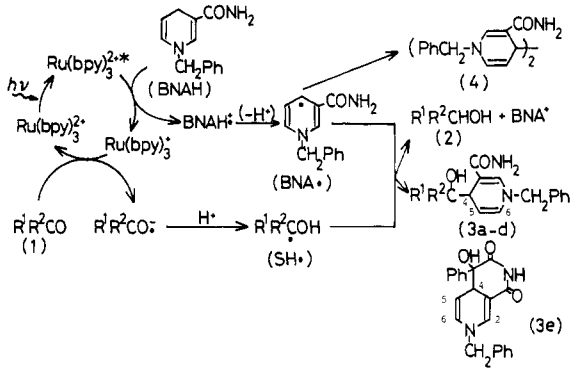
Deaerated metanolic solutions containing $Ru(bpy)_3Cl_2 \cdot 6H_2O$ (1×10^{-3} M), BNAH (0.1 M), and **1a–f** (0.05 M) were irradiated at >470 nm under cooling with water.[9] The progress of the reactions was followed by VPC, and the products were isolated by column chromatography on basic alumina; Table I summarizes the results. In cases **3a–d**, 1:1 mixtures of the diastereoisomers were obtained, from which each isomer was separated by repeated chromatography. Each of the isomers **3a**, **3b** and **3d** was isolated as a crystalline material while the other isomers and both isomers of **3c** remained oily. On the other hand, **3e** was isolated as a single crystalline isomer of the cyclic imide. In case **1f**, the reduction of **2f** quantitatively occurred without formation of any adduct. The structuers of **3a–e** were deduced from the spectroscopic properties.[10]

Table I. Product Yields in the $Ru(bpy)_3^{2+}$-Photosensitized Reactions of BNAH with 1a–f (R^1R^2CO)[a]

	R^1	R^2	time,[b] h	isolated yields, % 2[c]	3[c,d]	4[e]
1a	C_6H_5	H	15	0	85[f]	<1
1b	p-C_6H_4CN	H	20	16	56[g]	8
1c	2-pyridyl	H	20	20	33[h]	53
1d	C_6H_5	CF_3	15	<4	44[i]	9
1e	C_6H_5	CO_2Me	15	24	60[j]	9
1f	2-pyridyl	2-pyridyl	6	>90	0	50

[a] Methanolic solutions were irradiated at >470 nm under cooling with water (≤20 °C) where thermal reactions were negligible. [b] Irradiation time required for the complete disappearance of 1a–f. [c] Based on the 1a–f used. [d] Equimolar mixtures of the diastereoisomers except for 3e. [e] Based on the BNAH used. [f] mp 158.5–159 °C dec and oil. [g] mp 164.5–165 °C dec and oil. [h] Both isomers are oily. [i] mp 176.5–177.5 °C dec and oil. [j] A single isomer; mp 223–224 °C dec.

Scheme I



The luminescence of $Ru(bpy)_3^{2+}$ was quenched by BNAH[7] but not at all by **1a–f**. as discussed in a previous paper,[7] the photosensitized reactions are initiated by electron transfer from BNAH to excited $Ru(bpy)_3^{2+}$ followed by one-electron reduction of **1a–f** (Scheme I); $Ru(bpy)_3^{2+}$ can mediate one-electron transfer from BNAH to **1a–f** upon photoexcitation. The followup processes involve BNA· and HS· as intermediates which are formed by facile deprotonation of the cation radical of BNAH[11,12] and by protonation to the anion radical of **1a–f**, respectively, a

(1) Redox-Photosensitized Reactions. 10. Part 9: Pac, C.; Kubo, J.; Majima, T.; Sakurai, H. *Photochem. Photobiol.* **1982**, *36*, 273.
(2) Bruice, T. C. In "Progress in Bioorganic Chemistry", Kaiser, F. T., Kezdy, F. J., Eds.; Wiley: New York, 1976; Vol. IV, p 1.
(3) For a review, see: Eisner, U.; Kuthan, J. *Chem. Rev.* **1972**, *72*, 1.
(4) Tagaki, W.; Sakai, H.; Yano, Y.; Ozeki, K.; Shimizu, Y. *Tetrahedron Lett.* **1976**, *29*, 2541.
(5) Chipman, D. M.; Yaniv, R.; van Eikeren, P. *J. Am. Chem. Soc.* **1980**, *102*, 3244.
(6) van Eikeren, P.; Grier, D. L.; Eliason, J. *J. Am. Chem. Soc.* **1979**, *101*, 7406.
(7) Pac, C.; Ihama, M.; Yasuda, M.; Miyauchi, Y.; Sakurai, H. *J. Am. Chem. Soc.* **1981**, *103*, 6495.
(8) Sims, A. F. E.; Smith, P. W. G. *Proc. Chem. Soc.* **1958**, 282.
(9) A filter solution containing potassium chromate (30 g/L), sodium nitrate (300 g/L), and sodium hydroxide (10 g/L) was used in order to avoid direct photoexcitation of BNAH. It was confirmed that no reaction occurs in the absence of $Ru(bpy)_3^{2+}$ as well as in the dark.
(10) Sufficient analytical data were obtained for the crystalline compounds. Details of IR, UV, mass spectral, and ^{13}C NMR data that unambiguously support the assigned structures will be published in a full paper.
(11) Martens, F. M.; Verhoeven, J. W. *Recl. Trav. Chim. Pays-Bas* **1981**, *100*, 228.
(12) Blaedel, W. G.; Haas, R. G. *Anal. Chem.* **1970**, *42*, 918.

0022-3263/83/1948-2941$01.50/0