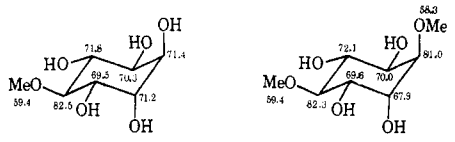
(10) Cf. also the shift data on pinitol (i) and pinpollitol (ii) [R. T. Gallagher, *Phytochemistry*, **14**, 755 (1975)]. (The authors acknowledge gratefully a gift of samples of these natural products.)



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Comparative Acidic Cleavage of Methoxybenzyl Protected Amides of Amino Acids[1]

P. G. Pietta,* P. A. Biondi,[2] and Oreste Brenna

Istituto di Chimica Organica dell'Università, 20133 Milano, Italy

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The use of 2,4-dimethoxybenzyl (Dmb) and *p*-methoxybenzyl (pmb) as amido protecting groups for conventional peptide synthesis has been previously reported.[3-5]

The possibility of using Dmb and pmb also in the solid-phase peptide synthesis[6] led us to study the relative stabilities of these groups in the acidic conditions normally associated with removal of the amino protecting groups.[7,8] For this purpose, cleavage of Dmb and pmb at 25° by 50% (v/v) trifluoroacetic acid in methylene chloride, 1 *N* HCl–acetic acid, and trifluoroacetic acid was investigated with the following protected amino acids: *N*-terbutyloxycarbonyl-N^{β}-*p*-methoxybenzyl and N^{β}-2,4-dimethoxybenzyl-L-asparagine [Boc-Asn(pmb)-OH and Boc-Asn(Dmb)-OH], *N*-*tert*-butyloxycarbonyl-N^{γ}-*p*-methoxybenzyl and N^{γ}-2,4-dimethoxybenzyl-L-glutamine [Boc-Gln(pmb)-OH and Boc-Gln(Dmb)-OH], and glycine-*p*-methoxybenzyl and 2,4-dimethoxybenzylamide (H-Gly-NHpmb and H-Gly-NHDmb). The removal of pmb and DMB was followed by thin layer chromatography. Partial cleavage of the amide protective groups was readily detected, since unchanged protected amides were well separated from L-asparagine, L-glutamine, and L-glycine amide resulting from cleavage.

In the case of H-Gly-NHpmb and H-Gly-NHDmb the removal of the protective groups was also checked by gas chromatography. Finally the action of liquid HF at 0° was examined[9] to ascertain whether pmb and Dmb had been removed under conditions where the side-chain protecting groups normally used in solid-phase peptide synthesis are cleaved.

This investigation has resulted in a method which is suitable to the quantitative determination of amino acid amides released during acidolysis of the corresponding *p*-methoxybenzyl or 2,4-dimethoxybenzyl amides. It should be noted that the results obtained by TLC are in good agreement with those obtained by GC (Table I).

The *p*-methoxybenzyl group was slightly affected by 1 *N* HCl–CH_3COOH under any conditions while prolonged exposure to this reagent caused partial cleavage of the 2,4-dimethoxybenzyl group (Table I).

Trifluoroacetic acid cleaved completely the 2,4-dimethoxybenzyl group after prolonged treatment (72 h), whereas the *p*-methoxybenzyl group was attacked partially only after 48 h (Table I).

2,4-Dimethoxybenzyl was completely removed by means of liquid HF; on the other hand, under these conditions the *p*-methoxybenzyl group was removed only partially. Boc-Gln(pmb)-OH, especially, has been shown to be sluggish to cleavage, confirming the work reported by Hruby et al.[10] Prolonged reaction times (12 h) were found necessary to complete removal.

These results demonstrate that 2,4-dimethoxybenzyl is suitable as an amido protecting group for asparagine and glutamine in solid-phase peptide synthesis and also indicate that *p*-methoxybenzyl is less promising in this connection.

Experimental Section

The Dmb derivatives were prepared as previously described.[5] The pmb derivatives were obtained in our laboratory as indicated below and their purity was checked by TLC using the following systems: A, benzene–ethyl acetate–petroleum ether (5:3:2 v/v); B, benzene–ethyl acetate–acetic acid–water (10:10:2:1 v/v); C, chloroform–methanol–acetic acid (15:3:2 v/v).

The removal of pmb and Dmb was followed by running chromatograms on Kieselgel G with 1-butanol–acetic acid–water (4:1:1 v/v); spots were detected with ninhydrin–cadmium acetate (0.2% v/v)[11] and evaluated by densitometry[12] using a chromoscan Zeiss double beam densitometer with thin layer attachment.

The gas chromatographic analysis was carried out using a Fractovap Model G.V. equipped with flame ionization detector. The

Table I
Stability[a] to Acidic Cleavages[b] of Dmb and pmb Amido Protecting Groups

Time, h	TLC/GC[c]		TLC			TLC		
	A	C	A	B	C	A	B	C
	H-Gly-NH-Dmb (35088-22-3)			Boc-Asn (Dmb)-OH (47553-91-3)			Boc-Gln (Dmb)-OH (31874-52-9)	
6	6/5	21/19		23	31		21	27
12	18/18.5	40/37		44	62		41	55
24	20/17.5	63/64	20	63	77	20	60	73
36	28/26	73.5/74	29	80	86	28	80	85
48	35/32	81/80	35	99	99	35	97	98
72	39.5/35	91/90	42	100	100	41	100	100
	H-Gly-NH-pmb (57459-57-1)			Boc-Asn (pmb)-OH (27482-66-2)			Boc-Gln (pmb)-OH (27482-68-4)	
48	3/2.6	26/28			13			15
72	6/5.5	35/35.5	5		20	3		21

[a] The percents of methoxybenzyl amides cleaved for different times are reported (mean of three independent determinations). [b] A, 1 N HCl–CH_3COOH; B, 50% CF_3COOH–CH_2Cl_2 (v/v); C, CF_3COOH. [c] Result of densitometric analysis/result of gas chromatographic analysis.

U-shaped glass column (2.5 mm × 2 m) was packed with 3% OV-101 on Gas-Chrom Q.

Nitrogen was used as carrier gas at a flow rate of 45 ml/min. Column temperature was kept constant at 210°.

***N*-*tert*-Butyloxycarbonyl-N^{β}-*p*-methoxybenzyl-L-asparagine Benzyl Ester [Boc-Asn(pmb)-OBzl].** This compound was prepared similarly to the Dmb analogue in 64% yield: mp 105–106° (from ethyl acetate); R_f(A) 0.74, R_f(B) 0.62.

Anal. Calcd for $C_{24}H_{30}N_2O_6$: C, 65.14; H, 6.83; N, 6.33. Found: C, 65.19; H, 6.87; N, 6.42.

***N*-*tert*-Butyloxycarbonyl-N^{β}-*p*-methoxybenzyl-L-asparagine [Boc-Asn(pmb)-OH].** Boc-Asn(pmb)-OBzl (2.21 g, 5 mmol) was hydrogenated in ethanol (50 ml) over 10% palladium on charcoal (0.6 g) for 10 h. The catalyst was then filtered off and the solvent evaporated. Crystallization from ethyl acetate gave the product (1.41 g, 80%): mp 132–133°; R_f(C) 0.71, R_f(B) 0.63; $[\alpha]^{20}D$ +9.92° (*c* 1.0, methanol).

Anal. Calcd for $C_{17}H_{24}N_2O_6$: C, 57.94; H, 6.86; N, 7.95. Found: C, 57.87; H, 6.84; N, 7.91.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-*p*-methoxybenzyl-L-glutamine Benzyl Ester [Boc-Gln(pmb)-OBzl].** This compound was prepared similarly to the Dmb analogue in 88% yield: mp 108–109° (from ethyl acetate); R_f(A) 0.80, R_f(B) 0.59.

Anal. Calcd for $C_{25}H_{32}N_2O_6$: C, 65.77; H, 7.06; N, 6.13. Found: C, 65.64; H, 7.10; N, 6.12.

***N*-*tert*-Butyloxycarbonyl-N^{γ}-*p*-methoxybenzyl-L-glutamine [Boc-Gln(pmb)-OH].** This compound was prepared similarly to the asparagine analogue in 94% yield: mp 97–98° (from ethyl acetate); R_f(C) 0.80, R_f(B) 0.72; $[\alpha]^{20}D$ −2.66° (*c* 1.0, methanol).

Anal. Calcd for $C_{18}H_{26}N_2O_6$: C, 59.00; H, 7.15; N, 7.64. Found: C, 59.06; H, 7.13; N, 7.68.

Action of 1 N HCl–Acetic Acid. The protected amides (0.1 mmol) were placed in test tubes and treated with 1 N HCl in CH_3COOH (1.5 ml). The tubes were stoppered and kept in a desiccator and the reaction was allowed to proceed for the required time at 25°. Then the reagent was evaporated under nitrogen and the residue was dissolved in 1 N HCl (100 ml).

Action of 50% Trifluoroacetic Acid–Methylene Chloride and Trifluoroacetic Acid. The protected amides (0.1 mmol) were placed in test tubes and treated with 50% trifluoroacetic acid in methylene chloride (v/v) or trifluoroacetic acid (2 ml) in the presence of anisole (0.2 ml) for the desired time at 25°. After the evaporation under nitrogen of the reagent the residue was quantitatively transferred to a 25-ml funnel with 1 N HCl and extracted twice with ether (5 ml each). The solution was diluted with 1 N HCl to a constant volume (100 ml).

Action of Hydrofluoric Acid. The protected amides (0.1 mmol) were treated with HF and anisole[7] for 1 h at 0°. The HF was then evaporated under nitrogen within 1 h and the residue was quantitatively transferred to a 25-ml funnel with 1 N HCl (10 ml). The solution was extracted twice with ether (5 ml) and then brought to 100 ml with 1 N HCl.

Densitometry. The spots were scanned at 490 nm and the areas under the densitometric curve were measured by the relationship area = peak height × width at half height. By reading from standard graphs for glycine amide, L-asparagine, and L-glutamine, the areas were related to the amounts of amino acid amide present.

Acidolysis and Gas Chromatography. H-Gly-NHDmb or H-Gly-NHpmb (0.5 mmol) were treated with trifluoroacetic acid (18.5 ml) and anisole (1.5 ml) or with 1 N HCl–CH_3COOH (20 ml), respectively. At fixed times portions of the reaction mixture were evaporated under nitrogen. The residue was treated with trifluoroacetic anhydride (0.5 ml) and methylene chloride (2 ml) for 30 min at room temperature. The solution was evaporated under nitrogen and the residue dissolved in ethyl acetate (0.5 ml) containing 0.2 mg of methyl stearate as internal standard; 1 μl was injected in the gas chromatograph. The retention times relative to methyl stearate for N-TFA-Gly-NHDmb and N-TFA-Gly-NHpmb were 1.11 and 0.72, respectively. The peak areas were calculated as peak height × width at half height and corrected against the peak area of the internal standard. The values of these areas were then related to the amounts of glycine amide produced at time *t* using the relationship $100 - (A_t/A_0)100$ where A_0 and A_t are the initial corrected area of N-TFA-Gly-NHDmb (or pmb) and that at time *t*, respectively.

Registry No.—Boc-Asn(pmb)-OBzl, 27482-84-4; Boc-Gln(pmb)-OBzl, 27482-67-3; HCl, 7647-01-0; acetic acid, 64-19-7; trifluoroacetic acid, 76-05-1; HF, 7664-39-3.

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Optically Active Amines. XXI.[1] Application of the Salicylidenimino Chirality Rule to Cyclic Steroidal Amines

Howard E. Smith,*[2a] Elizabeth P. Burrows,[2a] and Fu-Ming Chen[2b]

Departments of Chemistry, Vanderbilt University, Nashville, Tennessee 37235, and Tennessee State University, Nashville, Tennessee 37203

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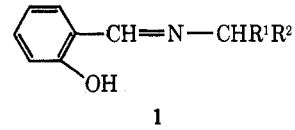
The salicylidenimino chirality rule[3] has been used to correlate the absolute configuration of *N*-salicylidenimino derivatives (1) of cyclic terpene amines (menthane, thujane,



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and fenchane ring systems) with the signs of the observed Cotton effects near 255 and 315 nm in their circular dichroism (CD) spectra.[1] The Cotton effects are generated by the coupled oscillator mechanism, demonstrated earlier to account for most of the observed optical activity in pyrimidine nucleosides.[4,5] For the *N*-salicylidene derivative the signs of the Cotton effects are determined by the chirality (right-handed screw for positive chirality) of vicinal carbon–carbon bonds and the attachment bond of the salicylidenimino chromophore.

We now apply similar consideration of a coupled oscillator mechanism to the interpretation of the CD spectra of the *N*-salicylidene derivatives of steroidal cyclic amines (Table I). These spectra were reported earlier,[6–9] but until now there has been no simple interpretation.

The electronic (isotropic) absorption (EA) spectra of *N*-salicylidene derivatives in hexane exhibit absorption bands at about 315 (log ϵ_{max} 3.68–3.73), 255 (log ϵ_{max} 4.12–4.21), and 215 nm (log ϵ_{max} 4.36–4.49),[1] designated as bands I, II, and III,[3] respectively, assigned to transitions of the intramolecularly hydrogen-bonded salicylidenimino chromophore[3] (2). In polar solvents such as dioxane, ethanol, and methanol a broad band at 400 nm (log ϵ_{max} 1.32–1.89 in dioxane[9] and log ϵ_{max} 3.06–3.38 in ethanol[9] and methanol[1]) and in ethanol a shoulder near 280 nm (log ϵ_{max} 3.49–3.67[9]) become evident, and the other three bands show a slight decrease in intensity.[1,9] The two additional bands are at-