A Convenient Synthesis of Benzyloxycarbonyl-L-amino Acid 4-Methylcoumaryl-7-amides

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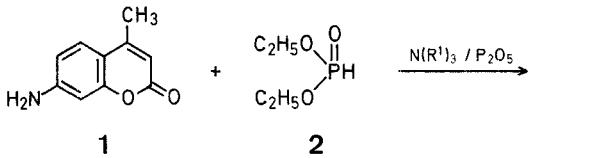
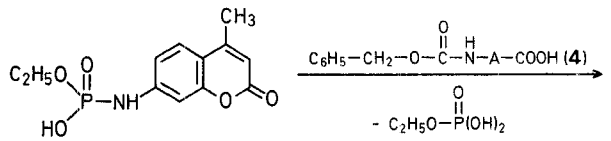
4-Methylcoumaryl-7-amides of amino acids or peptides have been employed as fluorogenic substrates for a variety of proteolytic enzymes[1-6]. In several cases these substrates gave more sensitive assays than were possible with analogous chromogenic substrates of the *p*-nitroanilide type due to the high fluorescence of the released 7-amino-4-methylcoumarin[1,3].

We were particulary interested in the *N*-α-benzyloxycarbonyl derivative of L-arginine 4-methylcoumaryl-7-amide (**5a**). This compound is not only an excellent substrate for trypsin and papain, but may be de-benzyloxycarbonylated and elaborated to give a variety of specific peptide substrates for diagnostically important enzymes. Examples of enzymes which cleave peptidyl-arginine 4-methylcoumaryl-7-amide substrates are α-thrombin, factor X_a, plasma kallikrein, urokinase, and plasminogen activator[4-6].

The synthesis of **5a** was reported by two groups using dicyclohexylcarbodiimide, with yields of 29%[3] and 13%[2], respectively. However, repeated attempts in our laboratory to obtain the material by the latter method or by mixed anhydride coupling failed to yield any product. In the search for alternative methods, we have found that a modification of the phosphorus pentoxide method of Schramm and Wissmann[7] gives the product in moderate yield with a minimum of purification necessary. This seldom-used coupling method involves an initial activation of the amine as a phosphoramide monoethyl ester **3**. The activated amine is then coupled with a carboxylic acid **4** to yield a carboxamide **5** as shown in the Scheme.



1 **2**



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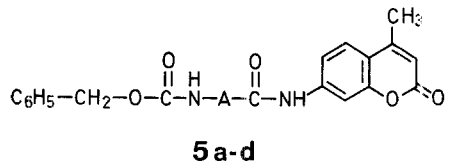

5a-d

R^1 = C_2H_5, n-C_4H_9;

5	A
a	$CH-(CH_2)_3-NH-C(=NH)-NH_2 \cdot HCl$
b	$CH-CH_3$
c	$CH-CH_2-C_6H_5$
d	$CH-C(=O)-O-CH_2-C_6H_5$ (CH$_2$–CH$_2$)

This method was also successfully employed for other benzyloxycarbonyl-amino acids **4b-d** to give the amides **5b-d** as optically active crystalline solids in yields ranging from 35–58%. The optimum conditions for reaction were found to be addition of 1 equivalent of 7-amino-4-methylcoumarin (**1**) and 1–2 equivalents of tertiary amine to a hot solution of phosphorus pentoxide in diethyl phosphonate (**2**; diethyl phosphite). After allowing for formation of the activated intermediate **3**, the protected amino acid **4** was added. The coupling was carried out for 1–2 h at 110 °C.

***N*-α-Benzyloxycarbonyl-L-arginine 4-Methylcoumaryl-7-amide Hydrochloride (5a):**

A suspension of 7-amino-4-methylcoumarin (**1**; 17 g, 0.09 mol) in diethyl phosphonate (**2**; 92 ml) containing triethylamine (12.5 ml, 0.09 mol) is added to a 110 °C solution of phosphorus pentoxide (25.5 g, 0.2 mol) in diethyl phosphonate (92 ml). After 20 min a 90 °C solution of benzyloxycarbonyl-L-arginine (**4a**; 27.7 g, 0.09 mol) in diethyl phosphonate (92 ml) containing 85% phosphoric acid (10.4 g, 0.09 mol) is added. After 2 h at 110 °C, the reaction is evaporated (60 °C/1 torr) to give an oil. The oily residue is stirred with 1 normal hydrochloric acid (910 ml) while heating at 90 °C. Upon cooling, the product crystallizes. After 18 h at 4 °C, the product is collected and dried. Further purification is accomplished by dissolving the product in boiling methanol (2500 ml), decolorizing with charcoal, filtering, and concentrating until incipient crystallization; yield: 23.4 g (52%); m.p. 213 °C (dec.); $[\alpha]_D^{20}$: −17.0° (DMF); Ref.[3] m.p. 210–211 °C; $[\alpha]_D^{20}$: −17°.

$C_{24}H_{26}ClN_5O_5$	calc.	C 57.43	H 5.62	N 13.95
(502.2)	found	57.39	5.64	13.87

Benzyloxycarbonyl-L-alanine 4-Methylcoumaryl-7-amide (5b):

In a good hood, a suspension of 7-amino-4-methylcoumarin (**1**; 21.2 g, 0.11 mol) in diethyl phosphonate (**2**; 100 ml) containing triethylamine (31.2 ml, 0.22 mol) is added to a 110 °C solution of phosphorus pentoxide (31.8 g, 0.22 mol) in diethyl phosphonate (100 ml). After 20 min at 110 °C, benzyloxycarbonyl-L-alanine (**4b**; 25 g, 0.1 mol) is added to the reaction suspension. Stirring is continued for 2 h at 110 °C, then the reaction mixture is evaporated (60 °C/1 torr) to give a semisolid residue. The residue is triturated with water (1000 ml), collected, and dried in vacuum. The crude solid is dissolved in hot, 1:1 chloroform/methanol (2500 ml) to give a turbid solution which is decolorized with carbon, filtered hot, and concentrated until incipient crystallization. After standing at 4 °C, the colorless product is collected, washed with hexane, and dried to give pure **5b**; yield: 25.2 g (57%); m.p. 219–220 °C; $[\alpha]_D^{20}$: −33.5° (DMF).

$C_{21}H_{20}N_2O_5$	calc.	C 66.31	H 5.31	N 7.36
(380.4)	found	66.53	5.20	7.21

Benzyloxycarbonyl L-phenylalanine 4-Methylcoumaryl-7-amide (5c):

Prepared as above for **5a** from benzyloxycarbonyl-L-phenylalanine (**4c**; 17.6 g, 0.06 mol). Product crystallizes upon concentration of a 1:1 chloroform/methanol solution; yield: 10 g (35%); m.p. 198–203 °C; $[\alpha]_D^{20}$: +56° (DMF).

$C_{27}H_{24}N_2O_5$	calc.	C 71.04	H 5.30	N 6.14
(456.5)	found	70.84	5.50	6.41

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Benzyloxycarbonyl-γ-L-glutamyl 4-Methylcoumaryl-7-amide α-Benzyl Ester (5d):

Prepared as above for **5a** from benzyloxycarbonyl-L-glutamic acid α-benzyl ester (**4d**; 21.5 g, 0.13 mol) except with tributylamine as base. The product is crystallized by concentration of a 1:1 chloroform/methanol solution; yield: 17.9 g (58%); m.p. 193–195 °C; $[\alpha]_D^{20}$: −22.5° (DMF).

$C_{30}H_{28}N_2O_7$	calc.	C 68.17	H 5.34	N 5.30
(528.6)	found	66.84[8]	5.65	5.37

Received: January 24, 1980

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[8] Result of only 1 analysis.

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