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Microwave-enhanced solution coupling of the α,α-dialkyl amino acid, Aib†

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Abstract—The difficult coupling of α-aminoisobutyric acid (Aib), during the synthesis of dipeptides (**1**–**6**), was carried out using PyBOP/HOBt and HBTU/HOBt reagents by application of microwave energy in the presence of solvent. Room temperature, conventional heating (oil bath) and microwave irradiation of the reactions are compared. Synthesis by microwave irradiation gave the desired compounds in higher yields and in shorter reaction times than those obtained by conventional heating or at room temperature.

α-Aminoisobutyric acid (Aib), the prototype of α,α-disubstituted glycines, is a non-coded amino acid found in membrane channel-forming peptides of microbial origin.[1] The presence of the extra-methyl group at the C^{α} atom dramatically restricts the conformational space accessible to Aib and forces the peptide chain into a left- or right-handed helical conformation.[2–7] Due to the stringency of the steric requirements, as well as to the high stability[8] and crystallizability[2] of its peptide derivatives, Aib has been often used to study the relationships existing between structure, stability and function in bioactive peptides,[9–11] long polypeptide chains,[12] and even proteins.[13] However, the incorporation of Aib into even short peptides has been strongly limited by poor yields in both solution and solid-phase synthesis using standard coupling reagents (i.e. DCC/HOBt), even for prolonged reaction times.[14,15] Other methods have been used, i.e. active esters,[16] mixed anhydrides,[17,18] but none of them gives consistently satisfactory results. Better results were obtained using BOP, PyBOP, PyBroP and BrOP.[19]

These limitations prompted us to explore novel experimental conditions, in order to improve Aib coupling efficiency and reduce reaction times. In recent years, the application of microwaves to organic synthesis has become increasingly popular,[20–27] by shortening reaction times and/or increasing yields. In the present paper, we report the results of a systematic study on the application of microwaves in the coupling of Aib to sterically hindered natural (i.e. Val, Ile) or non-coded (i.e. Aib) amino acids, using PyBOP/HOBt and HBTU/HOBt as coupling agents[28] (Table 1). Coupling yields and reaction times, obtained at room temperature and conventional heating (oil bath), were compared to those obtained by microwave irradiation.

The synthetic procedure by microwave irradiation was performed using a microwave oven (ETHOS 1600, Milestone) specifically designed for organic synthesis.[21] The results obtained with the coupling reagents PyBOP/HOBt and HBTU/HOBt are summarized in the Table 1, together with the experimental conditions employed. For compounds **1**–**4** we obtained high yields (76 to 87% in 1 h reaction at rt and 55°C, respectively)

Keywords: Aib; difficult couplings; microwave irradiation in solution.

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† Abbreviations and symbols follow the recommendations of the IUPAC-IUB Joint Commission on Biochemical Nomenclature (*Eur. J. Biochem.* **1984**, *138*, 9). In addition the following abbreviations are used: PyBOP, (1*H*-1,2,3-benzotriazol-1-yloxy)-tris(pyrrolidino)-phosphonium hexafluorophosphate; HBTU, *O*-benzotriazol-1-yl-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; BOP, (1*H*-1,2,3-benzotriazol-1-yloxy)-tris(dimethylamino)-phosphonium hexafluorophosphate; BroP, bromo-tris(dimethylamino)-phosphonium hexafluorophosphate; PyBroP, bromo-tris(pyrrolidino)-phosphonium hexafluorophosphate; DCC, dicyclohexyl-carbodiimide; HOBt, 1,2,3-benzotriazole-1-hydroxide; DIEA, diisopropylethylamine.

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Table 1. Comparison of the coupling yields for the synthesis of Aib-containing dipeptides at 23 and 55°C by conventional heating versus microwave irradiation[a]

Peptide	Rt (23 ± 2°C)				Conventional heating[b]				Microwave Irradiation[c]			
	PyBOP/HOBt		HBTU/HOBt		PyBOP/HOBt		HBTU/HOBt		PyBOP/HOBt		HBTU/HOBt	
	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (h)	Yield (%)	Time (min)	Yield (%)	Time (min)
1 Z-Aib-Val-OCH_3	80	1	74	16	84	1	77	16	96	30	94	30
2 Z-Val-Aib-OCH_3	82	1	35	16	87	1	45	16	94	30	95	30
3 Z-Aib-Ile-OCH_3	76	1	40	16	85	1	50	16	90	30	93	30
4 Boc-Ile-Aib-OCH_3	78	1	45	16	80	1	53	16	94	30	90	30
5 Z-Aib-Aib-OCH_3	84	16	86	16	86	16	90	16	92	30	90	30
6 Boc-Aib-Aib-OCH_3	80	16	50	16	88	16	60	16	88	30	86	30

[a] All dipeptides were synthesized by standard protocols,[27] unless otherwise specified. The reaction yields were determined by RP-HPLC analysis assuming a similar extinction coefficients at 220 nm for reagents and products. The values reported correspond to the average of the values obtained from three experiments under identical experimental conditions.

[b] Oil-bath at 55°C.

[c] Power 200 W for 15 min at 55°C and then 300 W for 15 min at 60°C.

using PyBOP/HOBt, while with HBTU/HOBt the yields were lower (35–77% for 1 h reaction at rt and 55°C, respectively). The results were identical whether Aib was in the C- or N-terminal position except for compound **1** when HBTU/HOBt method was used (both at rt or 55°C). Compounds **5** and **6** involved the difficult coupling of two Aib residues. For the HBTU/HOBt mediated coupling of N^{α}-protected Aib-OH to H-Aib-OMe (compounds **5** and **6**) the coupling yields varied significantly whether Z- or Boc-protecting groups were used. These differences were not observed when PyBOP/HOBt activation method was used. Instead, coupling under microwave irradiation of solutions gave, in any case, the desired dipeptides in higher yields (86–96% in 40 min) and cleaner reactions than those obtained by conventional heating or at rt. The overall reaction times were dramatically reduced, from 16 h to 30 min. Moreover, the coupling yields were not influenced by the particular activator employed (PyBOP and HBTU), or there were no appreciable differences in the coupling yields between the two coupling reagents, or by the N^{α}-protection (Z- or Boc) of the incoming residue (compounds **5** and **6**).

In conclusion, we have shown that the application of microwave irradiation improves coupling yields and significantly reduces reaction times in the solution synthesis of Aib-containing dipeptides. Our findings will be useful in the solution and possibly solid-phase peptide synthesis, when the incorporation of Aib or other sterically hindered amino acid residues into peptide chains is required.

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28. **Coupling Methods**: PyBOP/HOBt and HBTU/HOBt General procedure: To a DMF mixture of 1 mmol of *N*-protected acid component (Z- or Boc-Xaa-OH), 1.1 mmol of coupling reagent (HBTU/HOBt or PyBOP/HOBt), and 1.1 mmol of the *C*-protected amino acid (H-Xaa-OCH_3), 2 mmol of diisopropylethylamine (DIPEA, 3 mmol if amine salt was present) was added. The reaction was stirred at rt or by conventional heating or by microwave irradiation according to than reported in Table 1. The reaction mixture was separated from the solvent, the crude residue was taken up in ethyl acetate and washed successively three times with citric acid (5%), sodium bicarbonate (5%), and a saturated solution of sodium chloride. All dipeptides obtained were purified by preparative RP-HPLC and the homogeneity of the purified products was assessed by analytical RP-HPLC with a Vydac C18-column (5 μm, 4.6×250 mm, spherical). Analytical determinations were carried out by two solvent systems: A: 10%, (v/v), acetonitrile in 0.1% TFA, B: 60% (v/v), acetonitrile in 0.1% TFA (linear gradient from 100 A to 100% B over 25 min, UV detection at 220 nm, flow rate 1 mL/min). The final compounds were characterized by mass spectrometry (LCQ Thermoquest-Ion Trap) and the data were consistent with the considered structures.