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Microwave-assisted, zinc-mediated peptide coupling of *N*-benzyl-α,α-disubstituted amino acids

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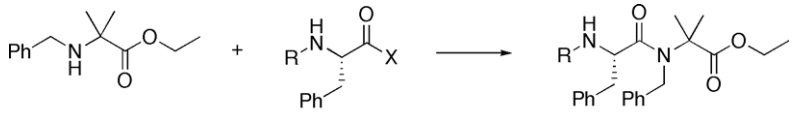
Abstract—Incorporation of the extremely hindered amino acid *N*-benzylaminoisobutyric acid into dipeptides under microwave irradiation with commercial zinc dust is described. A comparative survey of various methodologies for hindered peptide couplings was undertaken. The optimised coupling conditions were free from racemisation and applicable to other hindered amino acids.

The synthesis of peptides incorporating α,α-disubstituted and *N*-alkyl amino acids is an important process for the preparation of natural products and biologically active compounds.[1] Hence a wide variety of methods have been developed for both solution and solid phase synthesis of peptides incorporating such hindered amino acids. Fmoc-protected amino acid chlorides,[2,3] fluorides[4,5] and even bromides[6,7] have been used in preference to activated acids or mixed anhydrides as more activated partners for coupling to hindered residues. Further activation of amino acid halides has been achieved via sulfonamide protection in place of the less electron-withdrawing carbamate Fmoc group.[8,9] The use of sulfonamides has also been described to avoid cyclisation to easily racemised oxazolinones associated with carbamate protection. To enhance further the efficiency of hindered peptide couplings, activation of the amine by silylation, for example with *N*,*O*-bis(trimethylsilyl)acetamide (BSA), has been employed by various groups.[2,4]

We required a method to synthesise peptides containing an *N*-benzyl-α,α-disubstituted amino acid. Amongst the published methods, there is limited precedence for coupling with extremely hindered *N*-alkyl-α,α-disubstituted amino acids.[3,4,10] We sought to evaluate the applicability of relevant published methods to our system, using an *N*-benzyl-aminoisobutyric acid (Aib) derivative.

Various conditions were investigated and a selection is shown in Table 1. Coupling between the Aib derivative **1**[11] and the acid chloride derivative of **2** gave a higher yield of the dipeptide **3** than did the acid fluoride (Table 1, entries 1 and 2). Changing from Fmoc-Phe to the tosyl derivative, which has been reported to possess

Table 1. Conventional coupling conditions for the synthesis of 3[a]



1 **2** **3**

Entry	BSA (equiv)	Acid scavenger	R	X	Yield (%)
1	2	DIEA	Fmoc	Cl	29
2	2	DIEA	Fmoc	F	<10
3	2	—	Tosyl	Cl	20
4	10	Propylene oxide	Fmoc	Cl	6

[a] All reactions were carried out at room temperature with 4 Å molecular sieves and 1 equiv of acid scavenger (when used).

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enhanced reactivity[3] did not improve the yield (Table 1, entry 3). A significant decrease in yield was observed when propylene oxide was used as an acid scavenger in place of *N*,*N*-diisopropylethylamine (DIEA) (Table 1, entry 4).

During the course of our research, Babu and co-workers demonstrated coupling of α,α-disubstituted amino acids using commercial zinc dust under microwave irradiation, achieving high yields within short reaction times.[12] Initial investigations of microwave-assisted, metal-mediated peptide coupling on our system showed promise, but it was clear that optimisation was required to extend the methodology to the synthesis of the extremely hindered dipeptide **3**.

Using Fmoc-Phe-Cl and commercial zinc dust in dichloromethane, (Table 2), the coupling conditions were optimised by systematically varying temperature (entries 1–4), zinc concentration (entries 5–8) and reaction time (entries 9–13). An excellent conversion to **3** (81% as measured by LCMS) was achieved with the optimised conditions: 5 equiv zinc for 2 h at 90 °C (entry 12). No appreciable amount of premature deblocking of the Fmoc group was observed over the course of the reaction.

The effect of the choice of metal and solvent on the reaction was also investigated (Table 2). The degree of conversion was reduced for both less polar (toluene, entry 14) and more polar solvents (acetonitrile, entry 15) relative to dichloromethane. Furthermore, increasing the temperature in acetonitrile to 150 °C led to a dramatic reduction in conversion to **3** (entry 16). Increase in conversion to the dipeptide was not observed at temperatures above 90 °C in 1,2-dichloroethane (data not shown). Although no increase in dipeptide formation relative to zinc was observed with either magnesium (entry 17) or iron (entry 18), reasonable conversion with relatively clean reactions was afforded, indicating that there may be further scope for other metal-mediated peptide couplings. Using zinc (5 equiv) at reflux for 6 h without the microwave gave 67% conversion. Thus bench-top peptide couplings with zinc are viable, but they suffer from longer reaction times. As zinc is reported to neutralise hydrochloride amino acid salts,[2,13] it is conceivable that the zinc acts purely as a neutral acid scavenger in these reactions, although whether zinc has another role is still to be determined.

Table 2. Optimisation of microwave reaction conditions for the synthesis of **3**[14]

Entry	Temperature (°C)	M (equiv)	Time (min)	Solvent	% Conversion
1	40	Zn (2)	60	DCM	11
2	60	Zn (2)	60	DCM	22
3	80	Zn (2)	60	DCM	25
4	90	Zn (2)	60	DCM	41
5	90	Zn (0)	60	DCM	38
6	90	Zn (1)	60	DCM	42
7	90	Zn (5)	60	DCM	60
8	90	Zn (10)	60	DCM	56
9	90	Zn (5)	10	DCM	50
10	90	Zn (5)	30	DCM	60
11	90	Zn (5)	60	DCM	60
12	**90**	**Zn (5)**	**120**	**DCM**	**81**
13	90	Zn (5)	180	DCM	82
14	90	Zn (5)	120	Toluene	59
15	90	Zn (5)	120	MeCN	66
16	150	Zn (5)	120	MeCN	25
17	90	Mg (5)	120	DCM	36
18	90	Fe (5)	120	DCM	47

Table 3. Isolated yields of dipeptides prepared under optimised coupling conditions

Compound	Dipeptide	Yield (%)
3	Fmoc-Phe-*N*Bn-Aib-OEt	61
4	Fmoc-Ile-*N*Bn-Aib-OEt	40
5	Fmoc-Val-*N*Bn-Aib-OEt	54
6	Fmoc-Pro-*N*Bn-Aib-OEt	46
7[18]	Fmoc-Phe-*N*Me-Aib-OEt	80

Using the optimised coupling conditions (Table 2, entry 12), dipeptide **3** was prepared and isolated in 61% yield (Table 3), twice the best yield achieved with benchtop reactions. The optimised conditions were applied to the synthesis of other hindered dipeptides, incorporating α-amino acid residues with branching at the β-position (Table 3, compounds **4** and **5**), and the structurally constrained Pro residue (Table 3, compound **6**). It is noteworthy that the coupling proceeds cleanly and purification was considerably easier than when employing the methods in Table 1. Application of the optimised coupling conditions to the synthesis of a known dipeptide, Fmoc-Val-Aib-OBn,[15,16] afforded a product with matching optical rotation to the literature compound. This suggests that racemisation does not occur during the microwave reactions. Due to the prevalence of *N*-methyl amino acids in natural products and biologically relevant compounds,[1,17] the synthesis of compound **7** (Table 3) was carried out and the dipeptide was isolated in excellent yield.

The synthesis of extremely hindered dipeptides employing commercial zinc dust under microwave irradiation has been described. While the best conversion was achieved with zinc, encouraging results were also observed with iron and magnesium metals. The method is free from racemisation and the products are easily purified. This methodology could be applied to the synthesis of various peptides containing *N*-alkyl- and/or α,α-disubstituted amino acids.

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Supplementary data

Detailed descriptions of experimental procedures are included in the Supplementary data. Supplementary data

associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2007.06.109.

References and notes

1. Humphrey, J. M.; Chamberlin, A. R. *Chem. Rev.* **1997**, *97*, 2243–2266.
2. Babu, V. V. S.; Vasanthakumar, G. R.; Tantry, S. J. *Tetrahedron Lett.* **2005**, *46*, 4099–4102.
3. Carpino, L. A.; Ionescu, D.; El-Faham, A.; Henklein, P.; Wenschuh, H.; Bienert, M.; Beyermann, M. *Tetrahedron Lett.* **1998**, *39*, 241–244.
4. Wenschuh, H.; Beyermann, M.; Winter, R.; Bienert, M.; Ionescu, D.; Carpino, L. A. *Tetrahedron Lett.* **1996**, *37*, 5483–5486.
5. Carpino, L. A.; Beyermann, M.; Wenschuh, H.; Bienert, M. *Acc. Chem. Res.* **1996**, *29*, 268–274.
6. DalPozzo, A.; Bergonzi, R.; Ni, M. H. *Tetrahedron Lett.* **2001**, *42*, 3925–3927.
7. DalPozzo, A.; Ni, M. H.; Muzi, L.; Caporale, A.; de Castiglione, R.; Kaptein, B.; Broxterman, Q. B.; Formaggio, F. *J. Org. Chem.* **2002**, *67*, 6372–6375.
8. Carpino, L. A.; Shroff, H.; Triolo, S. A.; Mansour, E. M. E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 7829–7832.
9. Vedejs, E.; Lin, S. Z.; Klapars, A.; Wang, J. B. *J. Am. Chem. Soc.* **1996**, *118*, 9796–9797.
10. Dubuisson, C.; Fukumoto, Y.; Hegedus, L. S. *J. Am. Chem. Soc.* **1995**, *117*, 3697–3704.
11. The Aib derivative, **1**, was synthesized by first reacting Aib with thionyl chloride in ethanol to give the ethyl ester of Aib as the hydrochloride salt. This was then N-alkylated with benzyl bromide to give **1**.
12. Tantry, S. J.; Rao, R. V. R.; Babu, V. V. S. *Arkivoc* **2006**, 21–30.
13. Gopi, H. N.; Babu, V. S. *Tetrahedron Lett.* **1998**, *39*, 9769–9772.
14. In a typical procedure, **1** (0.20 mmol), Fmoc-amino acid chloride (0.20 mmol) and zinc dust (1.0 mmol) in dry dichloromethane (0.5 mL) were stirred at 90 °C for 2 h at 60–70 psi in a CEM Discover® microwave reactor. The reaction was cooled to room temperature, quenched with dry methanol (0.5 mL), filtered and concentrated onto silica. Purification by flash chromatography afforded pure products.
15. Fmoc-Val-Aib-OBn: $[\alpha]_D^{26}$ −18 (*c* 2, DCM), lit. $[\alpha]_D^{25}$ −20.6 (*c* 1, DCM).[16]
16. Babu, V. V. S.; Gopi, H. N. *Tetrahedron Lett.* **1998**, *39*, 1049–1050.
17. Wenger, R. M. *Angew. Chem., Int. Ed. Engl.* **1985**, *24*, 77–85.
18. *N*-Methyl-Aib-OEt hydrochloride (0.25 mmol), Fmoc-Phe-Cl (0.25 mmol), zinc dust (1.26 mmol) and DIEA (0.25 mmol) in dry dichloromethane (0.63 mL) were stirred at 90 °C for 30 min at ~65 psi in a CEM Discover® microwave reactor. The product was isolated as described previously.