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Incorporation of Cα-Methyl Amino Acids by Solid Phase Peptide Synthesis in a Peptide Sequence

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Abstract : (S)-αMethylmethionine, (S)-αMethylleucine, 2-aminoisobutyric acid and (S)-αMethylphenylalanine have been incorporated by solid phase peptide strategy in a peptide sequence. The coupling reactions of these Boc-αMe amino acids and of the following residue in the sequence were readily achieved after silylation with chlorotrimethylsilane of the amine function on the resin.

The need to develop amino acids with appropriate constraints around either Φ, Ψ and/or χ_1, χ_2 torsional angles became an evident need, as evidenced by literature (for recent reviews see[1, 2]). Indeed, restriction of either the inherent flexibility of a peptide backbone and/or the rotameric distribution of amino acids side chains has yielded agonists and in a few cases antagonists. Numerous peculiar amino acids that induced preferential secondary structures have been proposed. Among them, Cα-methyl amino acids are valuable tools for restricting the conformational mobility of a peptide backbone.[3] In short model peptides they promote helical structures[1,4] and 3_{10}-helical structures are mainly found with channel forming peptides containing α-aminoisobutyric acid (Aib) residues.[5] With the exception of Aib, α-methyl amino acids have rarely been introduced in the sequence of biologically active peptides to constrain their backbone mobility. The lack of commercially available enantiomerically pure α-methyl amino acids has probably hampered their implications in three dimensional structure activity relationships.

We recently reported[6] the diastereoselective synthesis of Cα-methyl amino acids, i.e. αMePhe, αMeMet, αMeLeu that we wished to incorporate in the sequence of Substance P (Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2), for stabilizing the postulated bioactive helical structure[7] of residues 4 to 8. Since both glutamine residues may be replaced by methionine and glycine by alanine[8], αMeMet and Aib can also be introduced in positions 5, 6 and 9, respectively. However, the first attempts to synthesize αMePhe-substituted SP analogues by automatic solid phase procedure proved to be quite a challenge. Indeed, even with a 5-molar excess, DCC/HOBt couplings yielded neither [αMePhe[7]]SP nor [αMePhe[8]]SP in satisfactory yield and purity. When α-MePhe and the following residue were manually introduced, reactions of both αMePhe[8] (on Gly[9]) and Phe[7] (on αMePhe[8]) were also sluggish whatever the conditions used, i.e., DCC/HOBt, DCC or BOP reagents[9,10], even with double couplings (2 x 5-fold-excess) and extended reaction times (overnight coupling). Bambino et al.[11] overcame the inefficient coupling of Aib or αMeSer, incorporating them by solid-phase synthesis as dipeptide units into a peptide sequence. However, this strategy imposes first the solution syntheses

+ Alié Brunissen died on June, 1st, 1996 after a car accident on September, 18th, 1995 and eight months of courageous fight.

of the various dipeptides, a tedious task. We found far more convenient and as efficient to activate by silylation the amino function on the resin before reacting with activated Boc-amino acid. Silylation has been previously used in amino acids prepararion as N-activating agent and/or C-protecting group.[12-14]

We first synthesized manually the C-terminal pentapeptide SP(7-11), (Boc strategy, 10-fold excess, DCC/HOBt coupling in NMP) with silylation[15] (10 equiv. chlorotrimethylsilane, CH_2Cl_2, 37°C, one hour) at positions 9 (NH_2 from glycine) and 8 (NH_2 from phenylalanine) prior to the next coupling. HF cleavage of the peptidylbenzhydrylamine resin showed that crude SP(7-11) was similar in terms of yield and purity to that obtained without silylation by automatic solid phase strategy (ABI Model 431A, 0.1 mmole scale, Boc strategy, DCC/HOBt coupling in NMP), only a slight increase in the percentage of SP(7-11) sulfoxides was noticeable.

Table 1. Comparison of Coupling Conditions for the Incorporation of α-Methyl Amino Acid into Peptide Sequences by Solid Phase Peptide Synthesis[a].

REAGENT	Gly-Leu-Met-Resin + Boc α-MePhe		Phe-Gly-Leu-Met-Resin + Boc α-MePhe	α-MePhe-Gly-Leu-Met-Resin + Boc Phe	
	(-)TMSCl	(+) TMSCl	(+) TMSCl	(+) TMSCl	(+) TMSCl[b]
DCC	43±9% (3)[c]	-	-	-	-
DCC/HOBt	55±8% (3)[c]	89±3% (2)[c]	88±3% (2)[c]	81±4% (2)[c]	79±5% (2)[c]
HATU	69±2% (2)[c]	83±6% (3)[c]	-	-	-

a: Coupling conditions: 100 mg resin (S = 0.38 to 0.52 meq), 3 equiv. Boc amino acid in NMP, 3 equiv. DCC or DCC/HOBt in 3 ml NMP or 3 equiv. HATU and 3 equiv. DIPEA in 3 ml NMP at room temperature for 3 hours, with a single coupling. For silylation: 10 equiv. chlorotrimethylsilane in CH_2Cl_2 for one hour at 37°C. b: In that case, silylation was performed at room temperature, i.e. 22 °C. After removal of the Boc protecting group (TFA/CH_2Cl_2, indol) and washing steps, coupling was quantified in duplicate on two different aliquots of resin (10-20 mg) by the Gisin test.[16] c: Results correspond to two or three (n) independent experiments.

Then, we analyzed the efficacy of silylation on the coupling of α-methylated amino acids (Table 1). HATU[17] (O-(7-azabenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate) activation notably increased the incorporation of Boc-α-MePhe when compared to DCC/HOBt coupling. However, DCC/HOBt and HATU activations were both efficient if the amino function was previously silylated (Table 1), therefore only DCC/HOBt activation was used for the next experiments. Steric hindrance of the amino function (Gly *vs.* Phe) did not alter the efficiency of Boc-α-MePhe coupling to Phe-Gly-Leu-Met-Resin after TMSCl activation of the NH_2 function (DCC/HOBt activation of the carboxylic function). Finally, coupling Boc-Phe to αMePhe linked on the resin was similarly successful if the amino function of αMePhe on the resin was activated by silylation. Silylation may also be performed at room temperature instead of 37°C as in the first experiments. Boc-α-MePhe-F was not employed, since we were unable to synthesize the fluoride derivative of Boc-α-MePhe, using cyanuryl fluoride.[18,19]

Thus, α-methylated analogues of SP listed in Table 2 have been prepared by this strategy, i.e., silylation[15] of the amino function on the resin prior to the reaction with Boc-α-Me-amino acid (DCC/HOBt coupling, 3 hours in NMP, room temperature, 5-molar excess). After removal of the Boc protecting group and neutralization, the amino function of the α-methylated amino acid was also silylated (TMSCl, 37°C) before reacting the following residue. These steps were done manually to allow control of the couplings.[10]

All these SP-methylated analogues were obtained with satisfactory yields and purities (yields[20] have not been optimized since purity was the only criterium). [αMeMet[6]]SP was obtained in a lower yield, a minor impurity (≤10 %) present in the crude product precluded a correct recovery. However, coupling of proline on α-MeMet led to [αMeMet[5]]SP with good yield and purity (33% yield, 98.2% pure). For comparison, the yield and physicochemical properties of SP are reported in Table 2.

Table 2. Yields and Physicochemical Properties of Substance P Analogues Incorporating α-Methyl Amino Acid.

Peptide[a]	Yield[b] (%)	HPLC[c] % Purity	HPLC[c] % CH_3CN	TLC[d] Rf	$[\alpha]_D^{19}$[e]
SP[f]	49	99.7	22.2	0.19	-81
[αMeMet[11]]SP[g]	63.5	99.4	23.4	0.16	- 73
[αMeLeu[10]]SP[g]	43.5	98.6	24.0	0.14	-72
[Aib[9]]SP[g]	34	99.9	26.4	0.12	- 63
[αMePhe[8]]SP[g]	20	96.7	25.8	0.14	- 79
[αMePhe[7]]SP[g]	21	97.6	26.4	0.14	- 76
[αMeMet[6]]SP[g]	8.5	94.0	27.6	0.17	- 49
[αMeMet[5]]SP[g]	33	98.2	26.4	0.17	- 67

SP = Substance P = Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

a : Molecular weight determined using MALDI mass spectrometry. b : Yield of the final product obtained after preparative HPLC purification and based on the amino substitution of the methylbenzhydrylamine resin, 0.1- or 0.15 mmole synthesis scale. c : Analytical HPLC ,% CH_3CN in 0.25 M TEAP buffer[6] pH 3.0. d : n-butanol-acetic acid-water, 4:1:5 (upper phase). e : Value for (c 0.46, 10 % acetic acid). f : SP: peptide synthesized on ABI Model 431A on MBHA resin, 0.1-mmole scale, 10-fold excess of Boc-amino acids, DCC/HOBt couplings. g: silylation[15], TMSCl 10 equiv., CH_2Cl_2, 37°C, one hour.

To our knowledge this letter reports the first direct silylation and activation of amino function on the resin for solid phase peptide synthesis. While this work was already submitted, we became aware of the work of Wenschuh et al.[21] describing the activation by silylation of hindered amino acids for coupling reaction in solution of Fmoc-amino acids fluorides. Now, further experiments are needed to extend this procedure to 1) hindered amino acids and "difficult sequences" and 2) to full automatic strategy.

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REFERENCES AND NOTES

1. Toniolo, C. ; Crisma, M. ; Formaggio, F. ; Valle, G. ; Cavicchioni, G. ; Précigoux, G. ; Aubry, A. ; Kamphuis, J. *Biopolymers*, **1993**, *33*, 1061-1072.

2. Hruby, V.J. In : *Peptides, Chemistry, Structure and Biology, Proceedings of the 13th American Peptide Symposium*, (Hodges, R.S. ; Smith, J.A. ; eds), **1994**, ESCOM, Leiden, pp 3-17.
3. Turk, J. ; Panse, G.T. ; Marshall, G.R. *J. Org. Chem.*, **1975**, *40*, 953-955.
4. Toniolo, C. ; Pantano, M. ; Formaggio, F. ; Crisma, M. ; Aubry, A. ; Bayeul, D. ; Précigoux, G. ; Dautant, A. ; Boesten, W.H.J. ; Schoemaker, H.E. ; Kamphuis, J. In : *Peptides, Chemistry, Structure and Biology, Proceedings of the 13th American Peptide Symposium*, (Hodges, R.S. ; Smith, J.A. ; eds), **1994**, ESCOM, Leiden, pp 315-316.
5. Karle, I.L. In : *Peptides, Chemistry, Structure and Biology, Proceedings of the 14th American Peptide Symposium*, (Kaumaya, P.T.P. ; Hodges, R.S. ; eds), **1996**, ESCOM, Leiden, in press.
6. Ayoub, M. ; Chassaing, G. ; Loffet, A. ; Lavielle, S. *Tetrahedron Lett.*, **1995**, *36*, 4069-4072.
7. Chassaing, G. ; Convert, O. ; Lavielle, S. *Biochim. Biophys. Acta*, **1986**, *873*, 397-404.
8. Lavielle, S.; Chassaing, G.; Julien, S.; Besseyre, J.; Marquet, A.; Beaujouan, J.-C.; Torrens, Y.; Glowinski, J., *Neuropeptides*, **1986**, *7*, 191-200.
9. Abbreviations : DCC : dicyclohexylcarbodiimide, HOBt : 1-hydroxybenzotriazole ; BOP : benzotriazole-1-yl-oxy-tris-pyrrolidinophosphonium hexafluorophosphate ; NEt_3 : triethylamine ; NMP : N-methylpyrrolidone; Aib : 2-amino isobutyric acid ; TEAP : triethylammonium phosphate buffer.
10. After Boc deprotection, the ninhydrin test of αMe amino acid is negative, coupling can be monitored with picric acid either qualitatively (yellow beads) or quantitatively (Gisin test extended to αMe amino acids).
11. Bambino, F. ; Brownlee, R.T.C. ; Chin, F.C.K. *Tetrahedron Lett.*, **1994**, 4615-4618.
12. Kricheldorf, H.R., *Liebigs Ann. Chem.*, **1972**, *763*, 17-38.
13. Bolin, D.R. ; Sytwu, I.I. ; Humiec, F. ; Meienhofer, J. *Int. J. Peptide Protein Res.*, **1989**, 33, 353-359.
14. Anteunis, M.J.O.; Belu, C. *Bull. Soc. Chim. Belg.*, **1987**, 96, 119.
15. The resin was treated with chlorotrimethylsilane (10 equiv. versus NH_2 substitution) in CH_2Cl_2, at 37°C, for one hour. After washings (CH_2Cl_2, NEt_3-CH_2Cl_2 1/9, CH_2Cl_2, NMP), the Boc-protected amino acid previously activated for 5 min with DCC/HOBt, 1 equiv. in NMP, was reacted for 3 hours at room temperature. A 5-molar excess of Boc-αMe amino acid and a 10-molar excess of non methylated amino acid were used.
16. Gisin, B.F. *Anal. Chem. Acta*, **1972**, *58*, 248-249.15.
17. Carpino, L.A.; El-Faham, A.; Albericio, F.; J. Chem; Soc., Chem. Commun., **1994**, 201.
18. Carpino, L.A.; Sadat-Aalae, D.; Chao, H.G.; DeSelms, R.H. *J. Am. Chem. Soc.*, **1990**, *112*, 9650-9652.
19. Similarly, Bienert et al. did not succeed in the preparation of Boc-α-Me amino acid-fluoride, personal communication.
20. Peptides were purified by preparative reverse-phase HPLC on a 10-x250- mm Brownlee column packed with Aquapore octyl 300 A° pore size. The separation was accomplished with various gradients in aqueous 0.1% TFA at a flow rate of 6 ml/min with UV detection fixed at 220 nm. Before pooling, the collected fractions were cheked for purity by analytical HPLC. The separation was accomplished on a Lichrospher 100 RP-8e column at a flow rate of 1.5 ml/min in isocratic mode (solvent A: 0.25 M triethylammonium phosphate buffer, pH 3.0, solvent B: 60% acetonitrile in solvent A), with UV detection fixed at 210 nm.
21. Wenschuh, H.; Beyerman, M.; Winter, R.; Bienert, M.; Ionescu, D.; Carpino, L.A. *Tetrahedron Lett.* **1996**, in press.

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