

Recent Developments in Microwave Enhanced Solid Phase Peptide Synthesis

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Introduction

Microwave energy has proven to be a useful tool for enhancing slow and difficult chemical reactions. Its application has been successfully applied to solid phase peptide synthesis (SPPS) and shown useful for the synthesis of a range of difficult peptides [1-8]. The N-terminal amino group and peptide backbone are polar and they constantly try to align with the alternating electric field of the microwave, this helps in breaking up the chain aggregation (Figure 1). Previous studies have investigated the effects of microwave on aspartimide formation and epimerization and offered optimized conditions for susceptible sequences to these well-known side reactions [9]. This study aims to build on previous work and offer further improvements for routine microwave SPPS protocols.

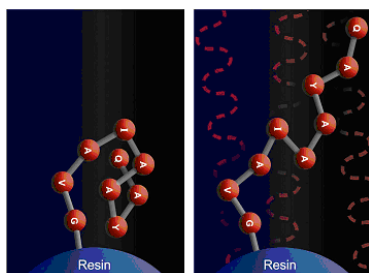


Fig. 1. Theoretical effect of microwave on aggregation

The focus was on optimizing the coupling conditions in microwave enhanced Fmoc SPPS of a series of reported very difficult peptides. The solvent, activator, and temperature of the coupling reaction were investigated. All peptides in this study were synthesized on a CEM Liberty automated microwave peptide synthesizer. Conventional synthesis results were obtained by programming the system to turn the microwave off during all steps.

Results and Discussion

The Arrhenius equation gives a measure of reaction rate based on temperature and activation energy E_a .

$$K = A e^{-E_a/RT}$$

Microwave energy interacts with molecular systems through both ionic conduction and dipole rotation that results in a kinetic excitation of the species. The kinetic excitation is observed by standard measurement that observes the average temperature of the system. Microwave energy interacts selectively with more polar components in a system which can lead to a wide range in molecular temperature at a given average system temperature. Larger differences in polarity between the reactants and solvent in a system will increase this effect. In systems where the solvent is less polar than the reactants, energy will be preferentially transferred to the reactants with the solvent acting as a heat sink. DMF, NMP, and DCM are commonly used solvents in peptide synthesis that vary in their polarity and ability to absorb microwave energy. While DMF and NMP are similar in microwave absorption, DCM is significantly less polar and absorbs less microwave energy.

The N- α -Fmoc- α -aminoisobutyric acid derivative (Aib) is a difficult derivative for coupling in SPPS due to its second methyl group on the α -carbon that interferes with the coupling reaction (Figure 2).

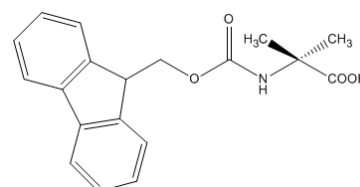


Fig. 2. N- α -Fmoc- α -aminoisobutyric acid

A sequence incorporating two consecutive Aib residues was selected for investigation due to its expected synthesis difficulties. Under conventional conditions with 30 min coupling time the purity was 8%, increasing the coupling time for Aib to 2 hours gave 19% purity, and by activator substitution to HCTU the maximum purity attainable was 30% in 38-hours. Under optimized microwave conditions, using HCTU activator and Aib amino acid solvent 50/50 DMF/DCM at a coupling temperature of 95 °C (10 min double coupling for Aib), the purity increased to 63% in 10-hours. Under these conditions, use of HBTU activator gave 54% purity. Thus, improvement in synthesis purity can be attributed to a combined beneficial effect of

DMF/DCM solvent mixture, reaction temperature of 95 °C and activator HCTU (Table 1).

Table 1. Synthesis of GEQKLG**AibAib**ASEEDLG-NH₂

Entry	Microwave	Activator	Synthesis Time (h)	Purity (%)
1	NO	HBTU	18.0	8
2	NO	HBTU	20.5	19
3	NO	HCTU	38.0	30
4	YES	HCTU	10.0	63
5	YES	HBTU	10.0	54

The use of higher temperatures allows for more microwave energy to be used to drive difficult reactions closer to completion. However, higher temperatures in peptide synthesis can be of concern due to possible epimerization [10,11]. To test the applicability of coupling temperatures at 95 °C, a previously used test peptide containing all twenty natural amino acids was selected for epimerization analysis with both HBTU and HCTU activation. As shown in Table 2 the coupling temperatures of cysteine and histidine were held at 50 °C due to their known epimerization at higher temperatures, while all other amino acids were coupled at 95 °C. The results indicate that using HBTU coupling temperatures of 95 °C did not increase epimerization. However, with HCTU increases in epimerization were observed with serine and tyrosine at 95 °C and with cysteine at 50 °C. This may be due to the higher acidity of the α -carbon proton that results from the electron withdrawing effect of the Cl atom unique to HCTU. This indicates that when using HCTU the maximum coupling temperature for natural amino acids should not be increased above 75 °C and coupling temperature for cysteine and histidine should be kept below 50 °C.

A mixed solvent system of DMF/DCM for the coupling reaction offers unique benefits under microwave conditions. The lower polarity of DCM allows for more of the microwave energy to be directed to the reactants and not the solvent. This allows for a greater amount of microwave energy to be introduced into a system at a given bulk temperature. Additionally, the boiling point of DCM is 40 °C, which is significantly less than the coupling temperatures of 50, 75, and 95 °C that were used. During the course of the reaction significant amounts of DCM will evaporate that increases the concentration of the reaction and may benefit the reaction.

Table 2. Racemization of amino acids measured by GC-MS after hydrolysis of VYWTSPFMKLIHEQCNRADG-NH₂ with 6N DCI/D₂O [12,13]

Amino acid (%)	HBTU in DMF (95 °C) ^a	HCTU in DMF (95 °C) ^a
D-Ala	0.27	0.28
D-Asp	1.08	1.71
D-Arg	0.27	0.29
D-Cys	1.11	1.57
D-Glu	1.37	1.48
D-His	1.36	4.46
D-Ile	0.10	0.10
L-allo Ile	0.10	0.13
D-allo Ile	0.15	0.18
D-Leu	0.28	0.41
D-Lys	0.15	0.20
D-Met	0.67	0.84
D-Phe	0.37	0.71
D-Pro	0.20	0.15
D-Ser	0.87	1.98
D-Thr	0.20	0.20
L-allo Thr	0.30	0.30
D-allo Thr	0.10	0.16
D-Trp	0.24	1.37
D-Tyr	0.25	0.54
D-Val	0.10	0.18

^a50 °C for cysteine and histidine

Two published difficult sequences have shown poor synthesis results under standard conventional conditions [14,15]. These sequences shown in Table 3 and 4 were synthesized under a variety of conventional and microwave conditions. In both cases the use of microwave provided benefits compared to conventional approaches for synthesis purity. Clear benefits were seen in changing the solvent conditions to 50/50 DMF/DCM in all cases, except Fmoc-His(Trt)-OH which would not dissolve at 0.2M under this condition.

Table 3. Synthesis of VTRYLTFNSKSVLQ

Entry	Microwave	Activator	Synthesis Time (h)	Purity (%)
1	NO	HBTU	8.5	27
2	NO	HBTU	16.5	51
3	NO	HCTU	16.5	56
4	NO	HCTU	36.5	58
5	YES	HBTU	7.5	86

Table 4. Synthesis of PKYLQNTLKLATGMRNVPEKQTT

Entry	Microwave	Activator	Synthesis Time (h)	Purity (%)
1	NO	HBTU	14.5	< 5
2	NO	HBTU	27	< 5
3	NO	HBTU	59.5	< 5
4	NO	HCTU	59.5	< 5
5	YES	HBTU	14.5	25
6	YES	HCTU	14.5	27
7	YES	HCTU	21	47

In summary, use of less polar solvents can increase microwave effects and offer synthesis benefits. Higher temperatures up to 95 °C are beneficial for sterically hindered couplings using HBTU and HCTU activation.

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References

1. Yu, H.-M., Chen, S.-T., and Wang, K.-T. (1992) *J. Org. Chem.*, **57**, 4784-4875.
2. Erdelyi, M. and Gogoll, A. (2002) *Synthesis*, **11**, 1592-1596.
3. Collins, J. M., Collins, M. J., and Steorts, R. C. (2003) *Poster Presentation at the 18th American Peptide Symposium, Boston, MA.*
4. Bacsa, B., Desai, B., Dibo, G., and Kappe, C. O. (2006) *J. Pept. Sci.*, **12**, 633-638.
5. Fara, M. A., Diaz-Mochon, J. J., and Bradley, M. (2006) *Tetrahedron Lett.*, **47**, 1011-1014.
6. Matsushita, T., Hinou, H., Fumoto, M., Kurogochi, M., Fujitani, N., Shimizu, H., and Nishimura, S.-I. (2006) *J. Org. Chem.*, **71**, 3051-3063.
7. Tantry, S. J., Rao, R. V. R., and Babu, V. V. S. (2006) *ARKIVOC*, **1**, 21-30.
8. Byk, G., Cohen-Ohana, M., and Raichman, D. (2006) *Biopolymers*, **84**, 274-282.
9. Palasek, S. A., Cox, Z. J., and Collins, J. M. (2007) *J. Pept. Sci.*, **13**, 143-148.

10. Han, Y., Albericio, F., and Barany, G. (1997) *J. Org. Chem.*, **7**, 4307-4312.
11. Angell, Y., Alsina, J., Albericio, F., and Barany, G. (2002) *J. Pept. Res.*, **60**, 292-299.
12. Racemization analysis of amino acids was performed by C.A.T. GmbH & Co. [Tuebingen, Germany]
13. Kemp, D. In *The Peptides: Analysis, Synthesis, Biology*, Gross, E., Meinhofer, J. (eds). Academic Press: New York, 1979, 315.
14. Novabiochem Innovations (2007):
http://www.emdbiosciences.com/SharedImages/novabiochem/04_07_innovE.pdf
15. Sampson, W.R. (1999) *J. Pept. Sci.*, **5**, 403-409.