USING ACETONITRILE AND TETRAHYDROFURAN INSTEAD OF N,N-DIMETHYLFORMAMIDE IN PEPTIDE BOND FORMATION

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Introdution

In our opinion, the practical implantation of the synthetic methodologies used for peptide bond formation is very conservative. Either they have its roots into the tradition or even they are leadered by the designers of automatic synthesizers. The solvent used for all the synthetic process is a paradigm of that asseveration. Thus, during the 60's CH_2Cl_2 (dichloromethane) was the reagent of choice for performing all solid-phase synthetic process. Then, DMF (*N*,*N*-dimethylformamide) and in less extension NMP (*N*-methyl-2-pyrrolidone) are the only solvents used. In fact and according to a survey which was done by other group, about 83% of peptide bond formation was achieved by using DCM or DMF as a solvent. [1] However and waiting for the final implementation of the REACH, the search of different solvents than DMF and NMP is mandatory, because the use of these two will be restricted after REACH implementation.

Previous reports were published about classification of solvents on environmental point view. According to these studies, DMF and NMP were classified under the undesirable solvent. On the other hand, THF (tetrahydrofuran) and ACN (acetonitrile) were classified under the usable solvent. In fact, they reported that ACN can be a good replacement for dipolar aprotic solvents such as DMF, NMP and also DMA (*N*,*N*-dimethylacetamide).[1]

Several years ago, one of us demonstrated that acetonitrile (ACN) could be a good alternative for DMF and NMP [2]. Herein, we are discussing a deeper study on the use of both ACN and THF in peptide synthesis, in both solid-phase and solution chemistries.

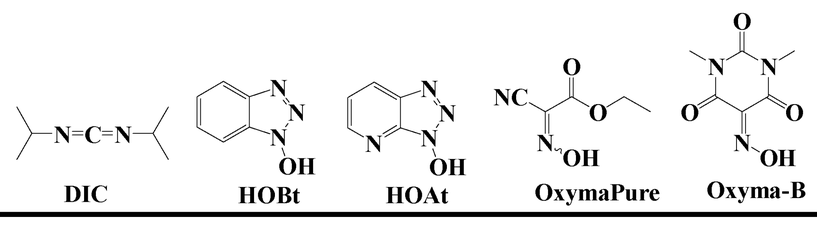

Table 1. Yield and racemization during the formation of Z-Phg-Pro-NH_2 (stepwise solution-phase synthesis).[a]

Entry	Coupling reagent	Solvent	Yield (%) [b]	DL/LL (%)[c]
1	DIC/HOBt	DMF	94.3	11.0
2		THF	93.3	8.7
3		ACN	94.4	4.3
4	DIC/HOAt	DMF	91.5	3.9
5		THF	93.9	2.3
6		ACN	93.9	2.7
7	DIC/OxymaPure	DMF	94.4	0.9
8		THF	93.5	0.6
9		ACN	95.7	0.6
10	DIC/Oxyma-B	DMF	90.0	1.0
11		THF	94.5	1.1
12		ACN	95.0	0.3

[a] Couplings were performed without preactivation at room temperature.[b] Conversion yield calculated by HPLC. Retention times of starting materials and products were identified by injection of pure sample. [c] Retention times for each epimer were identified after co-injection with a pure LL and DL samples.

Table 2. Yield and racemization during the formation of Z-Phe-Val-Pro-NH_2 (segment solution-phase synthesis).[a]

Entry	Coupling reagent	Solvent	Yield (%) [b]	LDL/LLL (%)[c]
1	DIC/HOBt	DMF	96.2	14.8
2		THF	92.2	4.8
3		ACN	96.5	10.5
4	DIC/HOAt	DMF	97.6	5.9
5		THF	94.3	0.9
6		ACN	96.8	1.7
7	DIC/OxymaPure	DMF	91.9	7.7
8		THF	91.8	1.9
9		ACN	96.1	0.7
10	DIC/Oxyma-B	DMF	90.7	5.1
11		THF	88.0	2.2
12		ACN	94.3	0.5

[a] Couplings were performed without preactivation at room temperature.[b] Conversion yield calculated by HPLC. Retention times of starting materials and products were identified by injection of pure sample. [c] Retention times for each epimer were identified after co-injection with a pure LLL and LDL samples.

Table 3. Racemization studies on the solid-phase assembling of H-Gly-Ser-Phe-NH_2 (stepwise solid-phase synthesis).[a]

Entry	Coupling reagent	Resin	Solvent	DL/LL (%)[b]
1	DIC/HOBt	PS	DMF	3.3
2		ChemMatrix	THF	0.3
3		ChemMatrix	ACN	0.4
4	DIC/HOAt	PS	DMF	0.4
5		ChemMatrix	THF	0.2
6		ChemMatrix	ACN	0.3
7	DIC/OxymaPure	PS	DMF	0.4
8		ChemMatrix	THF	0.2
9		ChemMatrix	ACN	0.3
10	DIC/Oxyma-B	PS	DMF	0.3
11		ChemMatrix	THF	0.2
12		ChemMatrix	ACN	0.3

[a] Couplings were performed 5 min preactivation at room temperature. [b] Retention times for each epimer were identified after co-injection with a pure LL and DL samples onto reverse-phase HPLC.

Table 4. Racemization studies on the solid-phase assembling of H-Gly-Cys-Phe-NH_2 (stepwise solid-phase synthesis).[a]

Entry	Coupling reagent	Resin	Solvent	DL/LL (%)[b]
1	DIC/HOBt	PS	DMF	0.5
2		ChemMatrix	THF	0.3
3		ChemMatrix	ACN	0.4
4	DIC/HOAt	PS	DMF	0.4
5		ChemMatrix	THF	0.2
6		ChemMatrix	ACN	0.3
7	DIC/OxymaPure	PS	DMF	0.3
8		ChemMatrix	THF	0.2
9		ChemMatrix	ACN	0.3
10	DIC/Oxyma-B	PS	DMF	0.3
11		ChemMatrix	THF	0.3
12		ChemMatrix	ACN	0.3

[a] Couplings were performed 5 min preactivation at room temperature. [b] Retention times for each epimer were identified after co-injection with a pure LL and DL samples onto reverse-phase HPLC.

Solid-phase peptide synthesis

Table 5. Percentage of tetrapeptide des-Aib (H-Tyr-Aib-Phe-Leu-NH_2) during solid-phase assembling of pentapeptide (H-Tyr-Aib-Aib-Phe-Leu-NH_2).[a]

Entry	Coupling reagent	Solvent	Penta (%)	des-Aib(%) [b]
1	DIC/HOBt	DMF	4.7	89.9
2		THF	9.6	90.4
3		ACN	12.4	89.9
4	DIC/HOAt	DMF	18.2	78.5
5		THF	45.9	54.1
6		ACN	53.6	41.5
7	DIC/OxymaPure	DMF	53.0	47.0
8		THF	93.6	6.4
9		ACN	91.8	8.2
10	DIC/Oxyma-B	DMF	19.6	80.4
11		THF	62.6	37.4
12		ACN	70.3	29.7

[a] Fmoc-RinkAmide-ChemMatrix resin and one hour coupling times were generally applied, except for Aib-Aib (one hour double coupling). [b] Deletion tetrapeptide (des-Aib) was identified by peak overlap in HPLC with an authentic sample obtained in solid phase.

Table 6. Solid-phase synthesis of Aib^{67}, Aib^{68}-modified ACP (H-Val-Gln-Aib-Aib-Ile^{69}-Asp-Tyr-Ile^{72}-Asn-Gly-NH_2).[a, b]

Entry	Coupling reagent	Solvent	Deca (%)	des-Aib(%)	Byproduct (%)
1	DIC/HOBt	DMF	8.2	38.6	42.1
2		THF	4.9	32.8	49.3
3		ACN	7.1	46.3	31.3
4	DIC/HOAt	DMF	23.8	53.0	14.2
5		THF	26.5	56.7	12.2
6		ACN	33.7	51.3	12.9
7	DIC/OxymaPure	DMF	37.8	34.0	21.9
8		THF	69.8	26.8	3.4
9		ACN	49.6	47.4	3.0
10	DIC/Oxyma-B	DMF	10.6	33.5	34.5
11		THF	59.7	10.7	18.3
12		ACN	47.3	43.3	2.4

[a] Fmoc-RinkAmide-ChemMatrix resin and one hour coupling times were generally applied, except for Aib-Aib (two hour double coupling). [b] Deletion tetrapeptide (des-Aib) was identified by peak overlap in HPLC with an authentic sample obtained in solid phase.

Acknowledgments

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Conclusions

- Either THF or ACN rendered the product in higher purity and less racemization level than DMF when using different peptide models in solution-phase or solid-phase peptide synthesis.
- Using OxymaPure with THF or ACN showed in most of the cases a superior racemization suppressor and then Oxyma-B.
- Using THF or ACN in solid-phase peptide synthesis in combination with a totally polyethylene glycol rendered the product in higher yield than DMF.
- Using THF or ACN in combination with OxymaPure gave the best result during solid-phase synthesis of some hindered peptide such as Aib-enkephaline pentapeptide and Aib-ACP decapeptide, then Oxyma-B more than HOAt. The worst result in this regard was HOBt.

References

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