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Peptide Synthesis in Fluorinated Alcohols Mixed with Proton Accepting Partners

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Abstract: *1,1,1,3,3,3-Hexafluoro-2-propanol is an excellent dissolver of protected oligopeptides but a barren medium for the condensation reaction of peptides. The solvent, however, could be changed to help the reaction proceed by mixing with a proton accepting solvent such as N,N-dimethylformamide.*

The solution-phase-peptide-synthesis often suffers from the solubility problem. The protected oligopeptide tends to lose its solubility to the common reaction media, such as dichloromethane and *N,N*-dimethylformamide (DMF), though it depends on the amino acid sequences. On the other hand, it is widely known that fluorinated alcohols, such as 2,2,2-trifluoroethanol (TFE) and 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP), are good solvents for poorly soluble protected oligopeptides.[1] The dissolving capacity of these alcohols is considered to owe to their strong hydrogen-bonding ability with carbonyl groups in peptide bonds. Accordingly, the utilization of TFE and HFIP has been examined in the solid-phase-synthesis (SPS) of a difficult-sequence peptide.[2] The mixed solvent of CH_2Cl_2 and HFIP (9/1, v/v) was effective to condition the peptidyl resin support. However, to our knowledge has not been reported the successful application of TFE or HFIP to the condensation of poorly soluble peptide fragments. Thus, aiming the application of HFIP to the solution-phase-peptide-synthesis (fragment condensation), we attempted to find out the appropriate reaction condition, in which the reactivity of HFIP to the reagents is suppressed.

Using the condensation reaction of Boc-Ala-OH (Boc, *t*-butyloxycarbonyl) and H-Ala-OBzl (Bzl, benzyl) by *N,N*-dicyclohexylcarbodiimide (DCC)[3] as a model system,[4] we have found out at first that TFE gave Boc-Ala-Ala-OBzl in moderate yield (73%) but HFIP did not (15%) (Entry 7 and 2 in Table 1, respectively). The reactivity of the OH group of HFIP may be responsible for the barren medium. Therefore, an aprotic polar organic solvent, DMF was examined for the effect to decrease the reactivity of HFIP to DCC. For the pairs of TFE/DMF and HFIP/DMF, the condensation efficiency was plotted to their ratios (Fig. 1). Obviously, the increasing amount of DMF to HFIP improved the yield more drastically than to TFE. The higher reactivity of HFIP toward DCC was supposed to be tamed by DMF with the hydrogen bond as illustrated in Fig. 2.

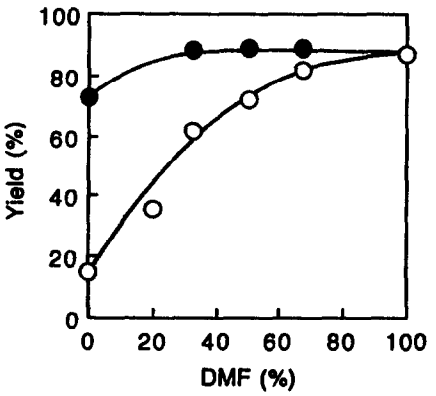

Fig. 1. Condensation yields in various DMF contents in HFIP (○) and TFE (●).

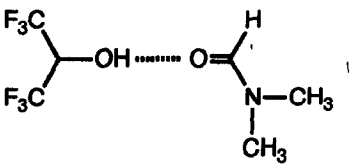

Fig. 2. Possible molecular pairing of HFIP and DMF with the hydrogen bond.

Table 1. The Condensation Yields in Various Mixed Solvents and by Different Reagents.[4]

Entry	Fluorinated alcohol	Proton acceptor	Ratio	Condensation reagent	Yield (%)
1	----	DMF	0/1	DCC	85
2	HFIP	----	1/0	DCC	15
3	HFIP	DMF	1/1	DCC	72
4	HFIP	DMSO	1/1	DCC	75
5	HFIP	pyridine	1/1	DCC	94
6	HFIP	CH_2Cl_2	1/1	DCC	52
7	TFE	----	1/0	DCC	73
8	TFE	DMF	1/1	DCC	89
9	TFE	DMSO	1/1	DCC	73
10	TFE	pyridine	1/1	DCC	99
11	TFE	CH_2Cl_2	1/1	DCC	85
12	HFIP	----	1/0	DCC/HOBt	17
13	HFIP	DMF	1/1	DCC/HOBt	98
14	HFIP	----	1/0	BOP/HOBt	73
15	HFIP	DMF	1/1	BOP/HOBt	89
16	HFIP	----	1/0	DPPA	3
17	HFIP	DMF	1/1	DPPA	37

The other aprotic polar and apolar solvents including dimethylsulfoxide (DMSO), pyridine, and CH_2Cl_2 were further examined for the capacity to protect the various condensation reagents. The results were summarized in Table 1. All the proton accepting solvents appeared to be effective to help DCC to produce Boc-Ala-Ala-OBzl in good yields (Entry 3-5 in Table1). Pyridine, which is not a good solvent for poorly soluble protected peptides, gave the highest yield. On the contrary, CH_2Cl_2 was less effective in the condensation (Entry 6) probably due to its very weak proton accepting capacity. The effect of added solvents, in the case of TFE, was repeated similarly as observed in HFIP (Entry 8-11).

Various condensation reagents were also examined about the influences of HFIP and the coexisting effects of the proton accepting partners (Table 1). The addition of 1-hydroxybenzotriazole (HOBt)[5] to DCC increased the condensation efficiency (Entry 13). It is noteworthy that the benzotriazole-1-yl-oxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP)/HOBt method[6] afforded 73% yield in HFIP alone (Entry 14). The proton accepting DMF could improved it to 89% (Entry 15). Diphenylphosphoryl azide (DPPA)[7] appeared not to be a good reagent in the condensation in HFIP containing media (Entry 16 and 17). Milton and Milton described their experiences with HFIP not to recommend in the formation of HOBt active ester of *N*-protected amino acid with DCC.[2] However, in this study the DCC/HOBt method could be utilized for condensation in HFIP mixed with DMF.

It should be emphasized that HFIP is a powerful dissolver of protected oligopeptides. Therefore, the use of HFIP in the fragment condensation of poorly soluble peptides will bring the great benefit in the chemical production of valuable hormonal peptides. In this aspect, the practical usefulness depends on the freedom from the racemization. The racemization was tested by the newly developed system, in which Boc-Ala-Ala-OH is coupled with H-Pro-$NHCH_3$ to give the diastereomeric tripeptide mixture. The condensation was carried out with the selected reagents and mixed solvents (Table 2). The corresponding diastereomers of Boc-Ala-Ala-Pro-$NHCH_3$ (LLL and LDL) are successfully separated by HPLC.[8] The DCC/HOBt method in HFIP/DMF (1/1, v/v) caused racemization as low as 3.9%. The mixed solvent of HFIP/DMF/pyridine (2/1/1, v/v/v) also appeared as a good medium with 1.6% racemization. However, the BOP/HOBt method was judged to be unsuitable for the fragment condensation, because it afforded 12% and 7.2% racemization in DMF alone and HFIP/DMF (1/1, v/v), respectively.

Table 2. Racemization during the Condensation in HFIP Containing Media.

Solvent	Condensation reagent	Racemization (%)
DMF	DCC/HOBt	1.9
HFIP/DMF (1/1, v/v)	DCC/HOBt	3.9
HFIP/pyridine (1/1, v/v)	DCC/HOBt	3.4
HFIP/DMF/pyridine (2/1/1, v/v/v)	DCC/HOBt	1.6
DMF	BOP/HOBt	12
HFIP/DMF (1/1, v/v)	BOP/HOBt	7.2
HFIP/pyridine (1/1, v/v)	BOP/HOBt	13
HFIP/DMF/pyridine (2/1/1, v/v/v)	BOP/HOBt	14

The utility of HFIP/DMF/pyridine (2/1/1, v/v/v) with DCC/HOBt was further demonstrated in the condensation of Ac-Glu(OcHex)-Leu-Leu-Lys(ClZ)-Ala-Pya-Ala-Glu(OcHex)-Leu-Leu-Lys(ClZ)-OH (a model peptide for the amphiphilic α-helix structure; *c*Hex, cyclohexyl; Pya, L-1-pyrenylalanine) with H-Ala-OBzl as follows. Though the acid component, 11-peptide, was hardly soluble in DMF, complete dissolution with HFIP was attained. The addition of DMF to a clear solution of the 11-peptide in HFIP resulted in partial gelation. This fact suggested that the hydrogen bonding of HFIP to the carbonyl group of the peptide was replaced to that of DMF. Therefore, some mixed solvents based on HFIP were examined for better dissolution. Finally, an equal volume mixture of DMF and pyridine was added to the HFIP solution of the 11-peptide. In the slightly turbid solution, the condensation reaction with H-Ala-OBzl afforded the product in 81% yield and >90% purity on HPLC. It was treated with anhydrous HF and analyzed by FAB-MS (m/z 1512 $(M + H)^+$). Thus, HFIP was shown as a useful co-solvent in the condensation of the peptide hardly soluble in DMF alone.

The solubility problem in the synthesis of peptide triggered the development of solid-phase-synthesis (SPS). However, SPS is difficult to be applied for the large scale synthesis of valuable peptides. The use of HFIP (or TFE if enough) by combination with a proton accepting partner as discovered in the present study may revive the solution-phase-synthesis.

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4. The condensation was carried out as follows. To a chilled solution of Boc-Ala-OH (1.0 mmol) and H-Ala-OBzl·*p*-toluenesulfonate (1.0 mmol) in the appropriate solvent (2.0 ml) was added the appropriate condensation reagent (1.5 mmol) and required triethylamine (1.0 mmol for DCC, 2.5 mmol for DPPA, and 3.25 mmol for BOP). The reaction was allowed to proceed for 5 h at room temperature. The product, Boc-Ala-Ala-OBzl, was extracted with 50 ml of ethyl acetate. After the *N, N*-dicyclohexylurea was filtered off if required, the solution was washed with 10% citric acid, 4% $NaHCO_3$, and water, successively, and dried over $MgSO_4$. After the solvent was removed by evaporation, the residues were dissolved in methanol (100 ml) and analyzed for Boc-Ala-Ala-OBzl by reversed-phase HPLC (YMC C18 column (4.6 x 150 mm) with a linear gradient of 10-100% acetonitrile/0.1% trifluoroacetic acid over 30 min).
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8. HPLC was carried out on a YMC C18 column (4.6 x 150 mm) with a linear gradient of 10-100% acetonitrile/0.1% trifluoroacetic acid over 30 min (detection, 220 nm; flow rate, 1.0 ml/min). The retention times of LLL and LDL isomers were 12.1 and 13.2 min, respectively.

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