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STUDY ON RACEMIZATION IN OXIDATION-REDUCTION CONDENSATION BY THE COUPLING REACTION FOR THE FORMATION OF BOC-LEU-ILE-ASP(NH_2)-LEU-OBU^t

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Racemization in the oxidation-reduction condensation was studied by the coupling reaction for the formation of Boc-Leu-Ile-Asp(NH_2)-Leu-OBu^t, recently reported for the detection of racemization in the azide, dicyclohexylcarbodiimide + 1-hydroxybenzotriazole methods, etc. The coupling reaction using triphenylphosphine and 2,2'-dithiodipyridine-1,1'-dioxide gave favorable results to the present racemization test on solid support as well as in solution synthesis.

It has been reported recently[1] that formation of D-alloisoleucine through coupling of Boc-Leu-Ile-OH and H-Asp(NH_2)-Leu-OBu^t in dimethylformamide (DMF) was employed for the detection of the racemization in azide, dicyclohexylcarbodiimide (DCC) + 1-hydroxybenzotriazole (HOBt), and other coupling methods.

In the present communication, suitable combination of oxidant and reductant in oxidation-reduction condensation was studied in this racemization test since a wide range of activation is possibly chosen by changing the nature of the oxidant or reductant as discussed in the previous report[2].

In the preceding reports, it has been shown that oxidation-reduction condensation by the use of triphenylphosphine (Ph_3P) and 2,2'-dipyridyl disulfide ($(PyS)_2$)[3] can be successfully applied to the syntheses of LH-RH[4] and ACTH(1-24)[5],[2] via fragment condensation on a solid support without accompanying any detectable racemization.

First of all, chain elongation from C-terminal amino acid to N-terminal amino acid (A type elongation) on solid support by coupling of Boc-Leu-Ile-OH with H-Asp(NH_2)-Leu-resin[6] containing 0.26 mmol/g of the dipeptide was tried in DMF under the conditions used in the Young test[7] but the coupling yield was 37% and extent of racemization[8] was 4.97%. Then the same reaction was tried at room temperature by using 2,2'-dithiodipyridine-1,1'-dioxide ($(Py(O)S)_2$)[9] in place of $(PyS)_2$ as an oxidant since it was assumed that the key intermediate of the reaction, phosphorane, would be able to keep the pentacovalent structure[2],[3] at temperatures higher than $-30^{\circ}C$ and favorable results were obtained as shown in Table I. In a typical experiment, 100 mg of H-Asp(NH_2)-Leu-resin, 3 eq each of Boc-Leu-Ile-OH,

$(Py(O)S)_2$ and 6 eq of 2-mercaptopyridine N-oxide (Py(O)SH) were suspended in 1 ml of DMF at 0°C. After stirring for a few min, 3 eq of Ph_3P was added and stirred for 2 hr at 0°C and additional 22 hr at room temperature and followed by the washing procedures[2]. The amino acid ratios of the resulting peptide-resin after hydrolysis with propionic acid - 12 N HCl[10] were as follows: Asp, 1.00; Ile + D-allo-Ile, 1.04; Leu, 1.98. These results show that coupling is quantitative and extent of racemization is 1.89%. This means that the extent of racemization during coupling reaction may be less than 1% since racemizations after hydrolyses of the peptide-resin of the stepwise synthesis and Ac-Ile-OH are 1.42% and 1.18% respectively. This procedure also gave favorable results in the case of a steric hindered amino component of H-Val-resin containing 0.12 mmol/g of Val and in the chain elongation from N-terminal amino acid to C-terminal amino acid (B type elongation) by coupling reaction of resin-Leu-Ile-OH[6] containing 0.25 mmol/g of the peptide with $H\text{-}Asp(NH_2)\text{-}Leu\text{-}OBu^t$.

Table 1. Racemization test in DMF by A type and B type chain elongation on solid support

	Coupling conditions[a]	Coupling yield, %	Extent of racemization, %
A type chain elongation	1. I	37	4.97
	2. I repeated 3 times	quant.	5.72
	3. II	quant.	1.89
	4. Coupling of Boc-Leu-Ile-OH with H-Val-resin by II	quant.	2.02
Blank	5. Stepwise synthesis[b]	quant.	1.42
B type chain	6. I repeated twice	~quant.	7.65
	7. II	quant.	2.02

a) I: 3 eq of carboxyl or amino component, Ph_3P, $(PyS)_2$ and 6 eq of PySH, 8 hr at -30°.
II: 3 eq of carboxyl or amino component, Ph_3P, $(Py(O)S)_2$ and 6 eq of Py(O)SH, 2 hr at 0°C and 22 hr at room temperature.

b) Starting with $H\text{-}Asp(NH_2)\text{-}Leu$-resin, Boc-Ile-OH and Boc-Leu-OH were successively coupled in CH_2Cl_2 by Ph_3P and $(PyS)_2$.

Next, these procedures were further studied on the peptide formation in solution and the results are summarized in Table 2.

When Ph_3P and $(PyS)_2$ were used as coupling reactants at -30°C, the reaction was slow and not completed within 10 hr and the procedure for decomposition[11] of the intermediate, phosphorane, was necessary since it increases reactivity at temperatures higher than -30°C causing racemization during the separation procedures at room temperature. This problem was solved by lowering the reactivity to carry out the coupling reaction at room temperature. A reaction using Ph_3P and $(Py(O)S)_2$ at room temperature also gave favorable results in solution.

Table 2. Preparation of Boc-Leu-Ile-Asp(NH_2)-Leu-OBu^t in DMF by solution synthesis

Reaction conditions	Yield, %	Extent of racemization,[a] %
1. Ph_3P + $(PyS)_2$ + 2 PySH, 10 hr at -30°C	43.3	10.2[b]
2. Ph_3P + $(PyS)_2$ + 2 HONB[c], 2 hr at -15°C, 5 hr at room temp.	70.2	3.59
3. Ph_3P + $(Py(O)S)_2$ + 2 Py(O)SH, 0.5 hr at 0°C, 5 hr at room temp.	78.2	1.82
4. Stepwise synthesis[d]	-	0.93
Azide method (Rudinger)	47.8[e]	1.4 ± 0.4[e]
" " (Curtius)	59.3[e]	11.2 ± 0.8[e]
DCC + HOBt	45.6[e]	7.5 ± 3[e]

a) Hydrolyses of samples were performed at the same time in 12 N HCl at 110°C for 24 hr.

b) Difference between this value and the value of 24.7 ± 2.1% reported in the literature[1] may be due to the lack of the decomposition of the remaining intermediate, phosphorane, in the latter case since experiment without decomposition procedure[11] gave 20.6%.

c) N-hydroxy-5-norbornene-endo-2,3-dicarboxiimide is known[1] as the best additive in DCC method to the present racemization test.

d) Starting with H-Asp(NH_2)-Leu-OBu^t, the tetrapeptide was prepared by coupling successively with Z-Ile-OH, Boc-Leu-OH by Ph_3P and $(PyS)_2$.

e) Data reported in the literature[1].

In a typical experiment, triphenylphosphine (0.5 mmol) in 0.5 ml of DMF was added at 0°C to a stirred mixture of 0.5 mmol each of Boc-Leu-Ile-OH, H-Asp(NH_2)-Leu-OBu^t, $(Py(O)S)_2$ and 1 mmol of Py(O)SH in 1 ml of DMF. After stirring for 30 min at 0°C and 5 hr at room temperature, the solvent was evaporated in vacuo and the residue was applied to Sephadex LH-20 in EtOH. Boc-Leu-Ile-Asp(NH_2)-Leu-OBu^t was obtained as a white solid after evaporation of solvent, 246 mg (78.2%): mp 205~6°, $[\alpha]_D^{20}$ -57.6° (c 2, MeOH). Found: C, 59.29; H, 9.19; N, 11.45. Calcd for $C_{31}H_{57}O_8N_5$: C, 59.30; H, 9.15; N, 11.16. Amino acid ratios are as follows: Asp, 1.00; Ile + D-allo-Ile, 1.05; Leu, 2.16; extent of racemization, 1.82%.

Based on these results, it is noted that the reactivity by use of Ph_3P and $(Py(O)S)_2$[12] is more effective to the suppression of the racemization in this racemization test than that of Ph_3P and $(PyS)_2$, though the question of which model for racemization is most similar to the practical peptide synthesis remains to be discussed. Soft[13] oxidants such as $(Py(O)S)_2$ and reductants with electron-withdrawing substituents such as tris-(p-bromophenyl)phosphite and pentafluorodiphenylphosphine increase the stability of the intermediate in the reaction and result in decreasing racemization. The order of the oxidants and reductants in terms of reactivity is summarized as follows: oxidant, X_2 (halogen)[14], RSX[15], $RS\text{-}NR'_2$[16], RS-OR'[17], $(PyS)_2$, $(Py(O)S)_2$; reductant, $(n\text{-}Bu)_3P$, $(MeO\text{-}C_6H_4\text{-})_3P$[18], Ph_3P, $Ph_2P\text{-}C_6H_4\text{-}SO_3H$[19], $(Br\text{-}C_6H_4\text{-}O\text{-})_3P$[20], Ph_2P-(pentafluorophenyl). These combinations provide a wide variety of reactivities allowing choice of a suitable one due to the variables as

the nature of carboxyl and amino component and reaction conditions such as temperature, solvent, concentration, etc, in the recently shown approach[2),4),5)] of solid phase peptide synthesis with monitoring technique by high-pressure liquid chromatography. Among these combinations, it should be noted from the standpoint of generality and availability that Ph_3P and $(PyS)_2$ are most common combination for usual peptide synthesis both in solution and on a solid support by either chain elongation via fragment condensation or stepwise synthesis. The use of $(Py(O)S)_2$ as oxidant in place of $(PyS)_2$ is effectively employed in fragment condensation reactions in DMF and the use of tri-p-anisylphosphine[20)] as reductant in place of Ph_3P promotes the coupling reaction in stepwise synthesis on solid support.

References and Notes

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