Tetrahedron Letters No. 3, pp 199 - 200, 1976. Pergamon Press. Printed in Great Britain.

RAPID SYNTHESIS OF OXYTOCIN ON THE SOLID PHASE USING ACTIVE ESTERS

Shabbir Ahmed Khan and K.M. Sivanandaiah*
Department of Chemistry, Central College, Bangalore University,
Bangalore 560001, India.

(Received in UK 13 October 1975; accepted for publication 4 December 1975)

Recently Konig and Geiger[1] observed that coupling reactions in peptide synthesis using active esters are greatly accelerated in the solution phase by the addition of 1-hydroxybenzotriazole (HOBt). That this acceleration takes place on the solid phase also is demonstrated by our rapid synthesis of oxytocin using active esters in the presence of HOBt. The synthesis was carried out in a solid phase peptide synthesis apparatus operated manually and the protected nonapeptide resin, Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-OCH_2-Resin, was obtained starting from Boc-Gly-OCH_2-Resin (containing 0.4 mM of Gly/g) by stepwise introduction of each amino acid residue using the appropriate active ester; the cycle of deprotection, neutralisation, and coupling reaction employed for each addition is shown in Table 1. The active esters

Table 1

Step	Reagent	Time (min)
1.	AcOH wash (3 times)	5
2.	(a) N HCl-AcOH	3
	(b) N HCl-AcOH	30
3.	AcOH wash (3 times)	5
4.	DMF wash (3 times)	5
5.	NEt_3-DMF (10%)	10
6.	DMF wash (3 times)	5
7.	3 eq. of Boc-amino acid active ester in DMF + 1 eq. of HOBt	As in Table 2
8.	DMF wash (3 times)	5
9.	EtOH wash (3 times)	5

For the removal of Boc group from Boc-Asn- and Boc-Gln-peptide resins substitute Steps 2 (a) & (b) with the following: (a) $BF_3 \cdot Et_2O$-AcOH[2] (10%) 45 min
(b) $BF_3 \cdot Et_2O$-AcOH (10%) 120 min

Table 2

Active ester	Reaction time (min)
Boc-Leu-OTcp[3]	55
Boc-Pro-OPcp[4]	60
Boc-Cys(Bzl)-OTcp[3]	60
Boc-Asn-OTcp[3]	90
Boc-Gln-OTcp[3]	55
Boc-Ile-OPcp[5]	210
Boc-Tyr-OTcp[6]	80
Z-Cys(Bzl)-OTcp[7]	30

employed and the durations of reactions are listed in Table 2. The completion of reaction in each step was monitored by the sensitive ninhydrin test described by Kaiser et. al[8].

The protected nonapeptide resin thus obtained was subjected to ammonolysis and the crude product was purified once from DMF-EtOAc to yield Z-Cys(Bzl)-Tyr-Ile-Gln-Asn-Cys(Bzl)-Pro-Leu-Gly-NH_2, m.p. 242-7°, $[\alpha]_D^{25°}$ -54° (c 1, DMF) (reported: m.p. 245-8°, $[\alpha]_D^{25°}$ -50.2° (c 1, DMF)[9]; m.p. 235-6.5°, $[\alpha]_D^{25°}$ -56.1° (c 1, DMF)[10]) in an overall yield of 24%. Reductive cleavage of the protecting groups from this peptide using sodium and liquid ammonia[11], followed by oxidation with $K_3Fe(CN)_6$ according to Hope[12] and purification on Sephadex G-15[13] furnished oxytocin in 64% yield. Thus in a typical experiment 66 mg of the protected nonapeptide yielded 32 mg of oxytocin having oxytocic activity of 510 IU/mg (reported: 500 IU/mg[9]; 480 IU/ mg[13]).

The salient features of this procedure are: 1) The shorter duration of the coupling reaction as compared with the prolonged period (4 to 18 h or even more)[4,14,15] that is necessary when active ester alone is employed. 2) The high degree of efficiency of the coupling reaction makes a single addition of 3 eq. (even in the case of hindered Boc-Ile-Opcp) suffice, as against the normal requirement of two additions each of 4 to 8 eq. of active ester[4,14,15].

The considerable economy of time and reagents thus achieved should extend the scope and utility of solid phase peptide synthesis.

Acknowledgement: We thank the authorities of the Drug Testing Laboratory of the Karnataka Government, Bangalore, for the bio-assay.

REFERENCES:

1. W. Konig and R. Geiger, Chem. Ber., 106, 3626 (1973).
2. E. Schnabel, H. Klostermeyer and H. Berndt, Liebigs Ann.Chem., 749, 90(1971)
3. W. Broadbent, J.S. Morley and B.E. Stone, J. Chem. Soc.,C, 1967, 2632.
4. B.J. Johnson, J. Pharm. Sci., 62, 1019 (1973).
5. B.J. Johnson and E.G. Trask, J. Org. Chem., 33, 4521 (1968).
6. Shabbir Ahmed Khan and K.M. Sivanandaiah, Unpublished work.
7. J. Pless and R.A. Boissonnas, Helv. Chim. Acta, 46, 1609 (1963).
8. E. Kaiser, R.L. Colescott, C.D. Bossinger and P.I. Cook, Anal. Biochem., 34, 595 (1970).
9. M. Bodanzsky and V. du Vigneaud, J. Am. Chem. Soc., 81, 5688 (1959).
10. K. Kuromizo and J. Meienhofer, J. Am. Chem. Soc., 96,,4978 (1974).
11. A.W. Myles and V. du Vigneaud, J. Med. Chem., 15, 11 (1972).
12. D.B. Hope, Experientia, 29, 389 (1973).
13. M. Manning, T.C. Wuu and J.W.M. Baxter, J. Chromatogr., 38, 396 (1968).
14. J. Roy, R.T. Haveran, I.L. Schwarz and R. Walter, Int. J. Pept. & Prot. Res., 7, 171 (1975).
15. B.J. Johnson and W.P. May, J. Pharm. Sci., 58, 1568 (1969).

*****