

ANDERSON TEST ASSESSMENT OF RACEMIZATION FOR THE HYDROXYBENZOTRIAZOLE-DICYCLOHEXYLCARBODIIMIDE PEPTIDE COUPLING PROCEDURE<sup>1</sup>

D.S.Kemp\*, M. Trangle, and K. Trangle  
Department of Chemistry, Massachusetts Institute of Technology  
Cambridge, Massachusetts 02139

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Since its introduction in 1969 by König and Geiger,<sup>2,3</sup> 1-hydroxybenzotriazole (HBT) has been widely recognized as an exceedingly useful racemization-suppressing additive for peptide coupling reactions with dicyclohexylcarbodiimide (DCC). Although these authors thoroughly investigated the extent of racemization under a variety of conditions,<sup>2</sup> using the model peptide couplings, BocLeuPheOH + ValOtBu, TfaProValOH + ProOtBu, and ZProValOH + ProOtBu, we felt that an application of our isotopic dilution variant<sup>4</sup> of Anderson's test, ZGlyPheOH + GlyOEt, would yield further pertinent data by permitting comparison of the HBT-DCC procedure with others for which Anderson data are available.<sup>4,5,6</sup> Table I lists our observations.

The levels of racemization observed for the HBT-DCC procedure span the range, 0.08-3 %, and are essentially those observed for N-hydroxysuccinimide-DCC couplings in DMF (0.2-0.6%) and for *p*-nitrophenyl esters in DMF (0.2-0.9%). All these procedures therefore fall in the middle racemization range--safe for many purposes, but not as secure as, for example, the azide method, which under optimum conditions leads to roughly an order of magnitude less racemization. This conclusion is in complete harmony with that reached by König and Geiger on the basis of their work with more hindered peptide models. Also in accord with their observations is the finding that variation of HBT concentration has only a small effect on racemate level.

Despite a random variation in the DMF data, it is clear that roughly a two-fold variation in DL yield occurs over a ten-fold change in reagent concentrations; a similar effect was observed for the NHS and *p*-NP ester cases reported previously<sup>6</sup>; all these variations can be rationalized in terms of a different molecularity in amine for coupling and racemization steps. The drop in yield with dilution in HMPA should be noted. The overall solvent racemization order is: HMPA > DMF > HMPA > DMSO > DMF > CH<sub>3</sub>CN > CH<sub>2</sub>Cl<sub>2</sub> > THF. DMF couplings at 0-2° give as low a DL level as is observed in THF, a somewhat surprising result in view of the 2-10 fold lower levels observed in THF for DCC-N-hydroxysuccinimide reactions.

Table I Anderson Test Data for the HBT-DCC Peptide Coupling Method

ZGlyPheOH + GlyOEt → ZGlyPheGlyOEt					
Solvent	Reagent Conc. M		Reaction Conditions <sup>a</sup>	Yield <u>L</u> %	Yield <u>DL</u> %
1. Variation of Reactant Concentration					
DMF	0.17		0-6° 1hr, 22° 20 hr	90	0.25
	0.08			89	0.32
	0.07			88	0.27
	0.05			94	0.32
	0.04			90	0.40
	0.03			92	0.35
	0.02			79	0.48
DMSO	0.33		2-6° 1 hr, 22° 20 hr	85	0.68
	0.17			84	0.74
	0.03			74	1.33
HMPA (hexamethyl- phosphoramide)	0.33		0-6° 1 hr, 22° 20 hr	96	0.97
	0.17			69	1.33
	0.03			29	1.43
DMF-HMPA (1:1,v/v)	0.17		0-6° 1 hr, 22° 20 hr	93	1.07
	0.03			53	3.01
2. Variation of HBT Concentration (Reaction time and temperatures as in 1.)					
DMF	0.17	1.1 equiv. HBT	1.1 equiv. GlyOEt	90	0.20
	0.17	1.3	1.1	91	0.17
	0.17	1.8	1.1	94	0.18
3. Variation of Temperature					
DMF	0.17		21 hr 22°	97	0.26
	0.17		21 hr 35°	91	0.27
	0.17		21 hr 0-2°	86	0.092
	0.17		1 hr 0-6°, 20 hr 35°	85	0.24
4. Other Solvents <sup>b</sup>					
CH <sub>3</sub> CN	0.3-0.03		0-6° 1 hr, 22° 20 hr	<u>    </u> <sup>b</sup>	0.11-0.16
CH <sub>2</sub> Cl <sub>2</sub>	0.3-0.03		0-6° 1 hr, 22° 20 hr	<u>    </u>	0.13-0.17
THF	0.3-0.03		0-6° 1 hr, 22° 20 hr	<u>    </u>	0.08-0.09

a. Unless otherwise specified, reactions were run by dissolving acid, amine, and HBT at 0° (1.0:1.0:1.2 equiv.) and adding 1.0 equiv. of DCC. GlyOEt was distilled before use. Solvents were reagent or spectro grade, distilled and stored over Molecular Sieves. Other reagents were prepared as described previously.<sup>4</sup>

b. Due to the cocrystallization of a radioactive impurity in the L product obtained from reactions in these solvents, accurate yields of L could not be calculated. The problem did not arise for DL samples, and racemate values were calculated assuming an overall yield of 100%; these values may be underestimated by 10-20%.

#### REFERENCES

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