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CYCLIZATION STUDIES OF TETRAPEPTIDE HOMOLOGS[1a]

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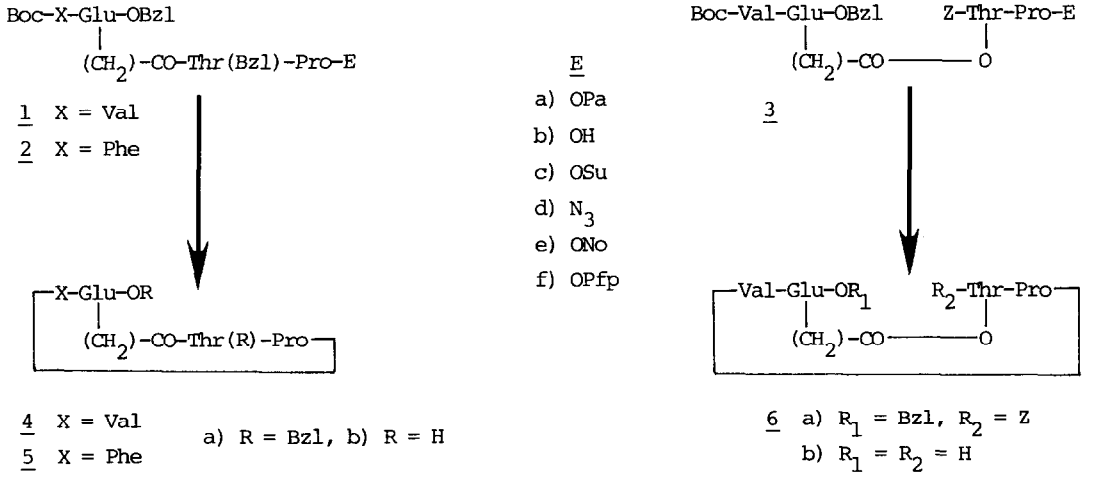
Abstract: A synthetic method of achieving enhanced yields of monomer cyclotetrapeptide homologs (all L configuration, 14 and 15 membered ring size) is reported.

As part of our continuing search for new agents which might be useful for the treatment of sickle-cell anemia,[2-5] we have synthesized two cyclic tetrapeptide homologs 4b and 5b and a tetrapeptide lactone homolog 6b. The intent was that these peptides would mimic a tetrapeptide region around the mutation site of hemoglobin S (HbS) and thus be able to bind at the acceptor site of HbS and thereby inhibit polymerization.[6]

The mono-cyclization of peptides is often difficult and is still regarded as an unsettled problem in organic chemistry, owing to the competition of cyclodimerization and polycondensation.[7,8] Our attempt at the preparation of 4a by the often used succinimide ester method,[7] or the azide method employing diphenylphosphoryl azide (DPPA)[9] at ambient or subzero temperatures, gave predominantly the cyclodimer, and the desired cyclomonomer 4a was present in only small amount (see Table 1).



It is known[9-11] that in the preparation of a given cyclic peptide the yield during the cyclization step is dependent upon the linear sequence. In those cases where cyclomonomer formation is disfavored the synthetic operations are varied, usually by trial and error, until the right linear sequence is found that gives acceptable cyclization yields. In order to avoid this time consuming trial and error method, we performed the cyclization of 1b (with Boc group removed) under elevated temperatures using different active esters, solvents, and catalyst conditions, in a modification of the method of Schmidt, et al.[11] (see Table 1). We found that cyclization of the Boc-deblocked 2-nitrophenyl ester 1e at 85° in the presence of 4-dimethylaminopyridine (DMAP, 0.80 equiv.) and CF_3CH_2OH (TFE, 1.5%) gave an increased yield of cyclomonomer 4a (18%) while reducing the amount of cyclodimer. When the cyclization was carried out with the pentafluorophenyl ester[11,12] 1f in DMF or dioxane in pyridine and varying the catalyst (Table 1) the yield of cyclomonomer 4a was increased up to 46%, while cyclodimer formation was almost entirely suppressed.[13] Similarly, cyclomonomer 5a could be obtained nearly free of cyclodimer by cyclization of the Boc-deprotected peptide ester 2f.

Table 1. [a]Cyclization Yields of Various Active Esters

Compd	Temp (°C)	[b]Solvent	Catalyst	% Yield Cyclo-Monomer	% Yield Cyclo-Dimer
1c	23	CH_2Cl_2/pyr	Nil	2	44
1d	-10	DMF/DIEA	Nil	3	27
1e	85	DMF/pyr	DMAP/TFE	18	trace[c]
1f	90	diox/pyr	PDP/EtOH	21	trace
1f	85	diox/pyr	DMAP/EtOH	37	trace
1f	85	DMF/pyr	DMAP/TFE	46	trace
1f	85	diox/pyr	DMAP	33	2
1f	85	diox/pyr	EtOH	43	6
1f	90	diox/pyr	Nil	31	13
2f	85	diox/pyr	DMAP/EtOH	19	trace
3f	85	diox/pyr	DMAP/EtOH	43	trace
3f	25-35	diox/pyr	DMAP/EtOH	small amount +	several side products

[a]Yields are based on 3 steps: ester formation, Boc-group removal, and cyclization, and are calculated from the purified products after chromatography. [b]diox = dioxane, pyr = pyridine. [c]Estimated to be less than 2%.

Maintenance of the temperature at 80-90° seems to be crucial. When the cyclization of the Boc-deprotected 3f was performed at slightly above room temperature considerable side products were formed in addition to a small amount of cyclomonomer 6a, whereas at 85° the cyclomonomer was formed in a 43% yield and only a trace of cyclodimer was observed (see Table 1). The catalysts DMAP[14] or 4-pyrrolidinopyridine (PDP)[11] do not increase the yield of cyclomonomer when compared to just EtOH or the absence of catalyst, however they appear to

suppress cyclodimer formation. The reason for the catalytic effect of alcohols (EtOH[11] or TFE) is not clear. The molecular weights of the cyclized peptides were established by high resolution fast atom bombardment mass spectrometry (FABMS).[13]

Since these cyclizations involved elevated temperatures in the presence of a basic solvent it was important to ascertain whether the L-configuration of the constitutent amino acids was retained during cyclization. To determine this we used the method of Shimohigashi, et al.,[15] which is a modification of the well known Manning and Moore[16] procedure. Thus, 4a was hydrolyzed (6N HCl/110°) and the liberated amino acids converted to their L-Leu dipeptides and identified by means of an amino acid analyzer[17]. We were only able to detect 1.3% of L-Leu-D-Pro and no L-Leu-D-Val or L-Leu-D-Thr. Under the conditions used we could not detect any L-Leu-D-Glu, however, a large peak for free Leu could have obscured it. Therefore we conclude that little or no racemization occurs under these cyclization conditions.

Our results indicate that linear peptides may be able to be cyclized preferentially to the monomer (without having to resort to sequence variation) by conducting the cyclization with pentafluorophenyl esters, elevated temperature, correct solvent, and addition of both an alcohol and DMAP as catalysts.

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References and Notes

1. a) Abbreviations generally follow the IUPAC-IUB recommendations or as slightly modified by E. Gross & J. Meienhofer, Eds., "The Peptides. Analysis, Synthesis, Biology", Vol. 2, Academic Press, New York, 1980, pp. XIII-XVIII.
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13. The tetrapeptide 1a was prepared by stepwise coupling of Pro-OPa with Boc-Thr(Bzl)OH, Boc-Glu-OBzl, and Boc-Val-OH by DCC/HOBt. TFA/CH_2Cl_2 (50%) was used to remove the Boc-group in each step. Selective removal of the Pa ester with Zn/AcOH afforded the free acid 1b. The latter acid was coupled with N-hydroxysuccinimide, 2-nitrophenol, and pentafluorophenol using DCC to give the esters 1c, 1e, and 1f, respectively. The azide 1d was prepared from 1b according to ref 9. The tetrapeptide 2f was prepared as above with the substitution of Boc-Phe for Boc-Val. The peptide 3a was obtained by coupling Z-Thr-Pro-OPa with Boc-Glu-OBzl using DCC/DMAP to form the ester. Removal of the Boc group and coupling with Boc-Val-OH gave 3a. Selective removal of the Pa ester and esterification with Pfp gave 3f. In all cases (except 1d) the active esters were treated with 50% TFA/CH_2Cl_2 and the resulting TFA salts in CH_2Cl_2, DMF, or dioxane were injected via a syringe pump over 16-18 h into a solution of the corresponding solvent and pyridine (20%), alcohol (1-1.5%), and catalyst (DMAP or PDP, 0.8-1 equiv.) maintained at a given temperature. The final dilution was 0.5mM. The crude product was purified by silica gel chromatography. 4b, 5b, and 6b were obtained by catalytic hydrogenation (Pd-C in TFE) of 4a, 5a, and 6a, respectively. 4a and 6a were further purified by HPLC (μBondapak C-18, 0.78 x 30 cm, 50% CH_3CN/H_2O). 4b, 5b and 6b were purified by HPLC (same column, 17-25% CH_3CN/H_2O). Compound: mp, $[\alpha]_D^{25}$, FABMS calcd (found). 4a: 216-218°, -35.1° (c 0.8, $CHCl_3$), 607.3132 (607.3122). 5a: 231-233°, -58.5° (c 1.1, $CHCl_3$), 655.3133 (655.3149). 6a: 74-77°, -39.4° (c 2.0, 20% MeOH/EtOAc), 651.3030 (651.3028). 4b: 238-241°, -72.7° (c 0.9, 25% CH_3CN/H_2O), 427.2193 (427.2203). 5b: 217-220°, -101.7° (K salt, c 0.8, H_2O), 475.2192 (475.2198). 6b: 229-231°, -53.5° (c 0.4, TFE), 427.2193 (427.2211).
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17. Analyses were performed on a Dionex D-500 amino acid analyzer, using their standard column, and detection was by ninhydrin. 0.2M Na citrate buffers of pH 3.25 and 3.78 were used. A mixture of the Boc-derivatives of Glu, Pro, Thr, and Val were hydrolyzed and converted to their L-Leu dipeptides in the same manner as 4a; no racemization due to the hydrolysis was detected. DL-Pro and DL-Val were also converted to their L-Leu dipeptides and used as controls.

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