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SYNTHESIS OF TYROCIDINE A: USE OF OXIME RESIN FOR PEPTIDE CHAIN ASSEMBLY AND CYCLIZATION

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Summary: Application of the Kaiser oxime resin to the synthesis of the thirty-membered ring cyclo-decapeptide Tyrocidine A (TA) is described. Assembly of the linear peptide chain and its subsequent cyclization with concomitant cleavage off the solid support were both achieved in high yield (73.2 % and 55 %).

We have recently developed a method for peptide cyclization on oxime resin (PCOR) that has been applied to the synthesis of diketopiperazines, cyclic tetrapeptides and a Lys^{i}, Glu^{i+4} side-chain bridged pentapeptide (21-membered ring)[1,2]. Tyrocidine A[3,4] (TA), a cyclo-decapeptide antibiotic (30-membered ring), has been selected as a synthetic target to demonstrate the applicability of this method to syntheses of larger main-chain cyclized peptides.

TA is generally produced by fermentation procedures (*Bacillus brevis*). The first synthesis of the open chain sequence was carried out in 1960 by R. Schwyzer *et al.*[5] The first total synthesis of TA was described only in 1966 by N. Izumiya *et al.*[6] Chemically synthesized TA and its analogues have usually been prepared in solution phase by step-wise assembly of the peptide chain, followed by an active ester ring closure step.[6-8]

In our solid-phase synthetic approach (Scheme 1) the *p*-nitrobenzophenone oxime polymer developed by DeGrado and Kaiser[9] was used as the solid support. Peptidyl oxime ester linkages to this resin have high acid stability, but are labile to aminolysis. The starting compound, Boc-Leu-resin (0.114 mmol Leu/g resin), was prepared from oxime resin having a significantly higher level of functionalization (0.544 mmol oxime/g resin), and the excess oxime groups were capped by acetylation. (The low peptide subtitution level on the solid support was utilized as a pseudo-dilution, in order to minimize interchain side-reactions during the cyclization step.) The peptide chain was then assembled by consecutive addition of the following N^{α}-Boc-amino acids: BocOrn(Z)OH, BocValOH, BocTyr(2,6-Cl_2-Bzl)OH, BocGlnOH, BocAsnOH, Boc-D-PheOH, BocPheOH, BocProOH and Boc-D-PheOH according to the BOP peptide coupling procedure.[10]

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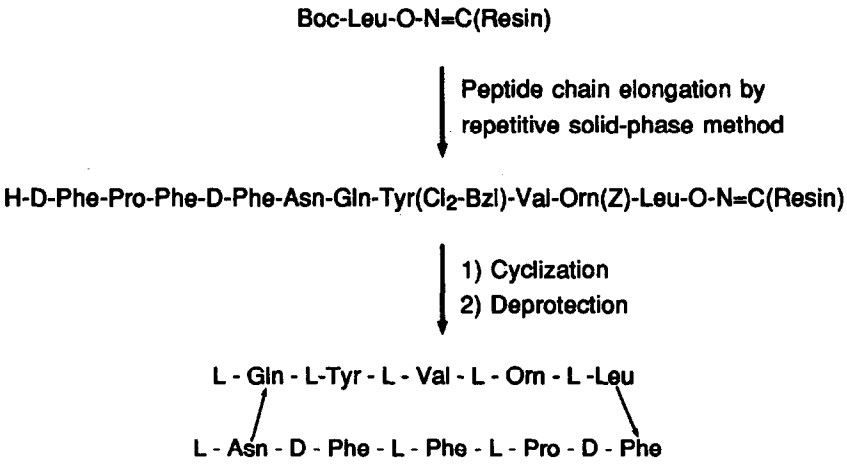

Scheme I: Tyrocidine A (TA) synthesis

Boc protecting groups were removed by treatment with 25 % TFA/DCM solution for 30 min. After the appropriate washing steps, Boc-amino acids and BOP reagent were added in 5-fold excess in DMF solution followed by the same excess of DIEA. After 2 hr reaction time the completeness of couplings was monitored by the Kaiser-test[11]. Couplings of BocAsnOH and BocGlnOH were repeated with 2.5 molar equivalent reagents. Yields of the coupling steps during the chain elongation are listed in Table I.[12]

After final removal of the Boc protecting group from the N terminus, the amino group was liberated from its TFA salt by addition of DIEA (1.5 equivalents). The free amino group cleaved the peptide from the polymer support by intrachain aminolysis in DCM at room temperature.[13] After 24 h reaction time, the product was obtained from the solution phase by filtration. This crude product was purified by silica gel chromatography (2 x 20 cm, eluent $CHCl_3$/MeOH/AcOH = 18/1/1). Yield 55%, based on the Leu content of the peptidyl resin; R_F(A) = 0.83. Amino acid analysis: Asn(1) 1.12, Gln(1) 1.13, Leu(1) 1.00, Orn(1) 0.85, Pro(1) 1.15, Phe(3) 3.18, Tyr(1) 1.01, Val(1) 0.99. Molecular mass by FAB-MS = 1563 (Calc. = 1562.7). Analytical RP-HPLC of this compound (Vydac C_4 Proteins analytical column; eluent system: 0.1% (v/v) TFA/acetonitrile-water with a linear gradient of 0-100% (v/v) acetonitrile over 45 min.) indicated >95% purity of the main component eluting at 66.6% (v/v) acetonitrile.

Protecting groups of the peptide were removed with TMSOTf in TFA in the presence of thioanisole.[14] Hydrolysis of the partly silylated product by NH_4OH was followed by gel permeation chromatography on Sephadex G-10 column (eluent: 2 M acetic acid in H_2O/MeOH, 4/1 [v/v]). Final purification was carried out by RP-HPLC on a Vydac C_{18} Proteins semi-preparative column eluted at 4 mL/min.with a linear gradient of 25-80% acetonitrile in 0.1% (v/v) TFA over 45 min. The product eluted as a symmetrical peak at 57% (v/v) acetonitrile. Yield 55%; R_F(B) = 0.83 (Lit.[6] = 0.76). Amino

Table I

Yields of coupling steps during peptide chain elongation

Coupled amino acid	Substitution level[a] (μmol/g)	Peptide (mmol)	Yield (%)
BocLeuO-resin	114.0	0.292	-
BocOrnOH	112.2	0.288	98.6
BocValOH	108.6	0.280	97.2
BocTyr(Cl_2-Bzl)OH	101.8	0.266	95.0
BocGlnOH	90.6	0.237	89.1
BocAsnOH	87.4	0.229	96.7
Boc-D-PheOH	86.2	0.227	99.1
BocPheOH	85.8	0.227	99.8
BocProOH	82.2	0.219	96.5
Boc-D-PheOH	80.2	0.214	97.7
Overall yield			73.2

[a]Based on Leu content by amino acid analysis of hydrolysates of the peptidyl resins.

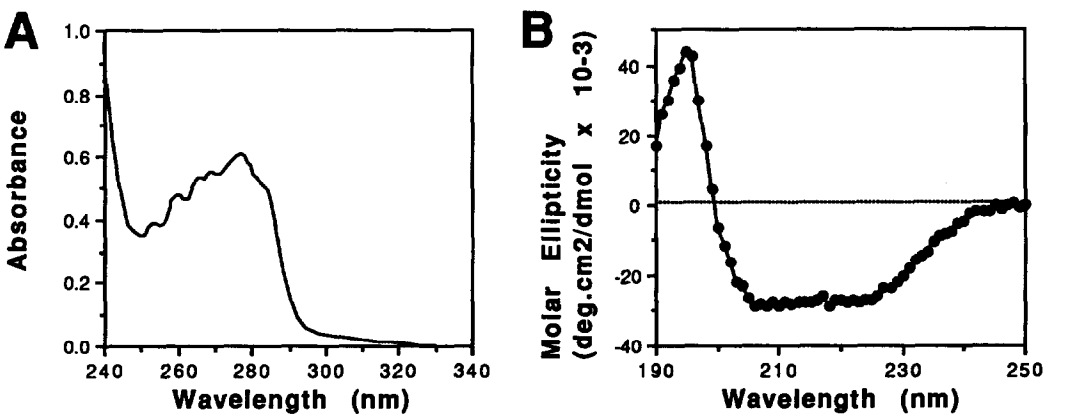

Figure 1: (A) UV spectrum of synthetic tyrocidine A (2.73×10^{-4} M) in EtOH/H_2O (1/1), λ_{max} = 278 nm. (B) CD spectrum of tyrocidine A (3.48×10^{-5} M) in EtOH/H_2O (1/1) measured using an Aviv Model 62ds spectropolarimeter and a 1 mm pathlength cell. Peptide concentrations were determined using ε_{278} = 2470 $M^{-1}cm^{-1}$ (calculated from the published UV spectrum[6]).

acid analysis: Asn(1) 0.98, Gln(1) 1.07, Leu(1) 0.93, Orn(1) 0.83, Pro(1) 0.92, Phe(3) 3.00, Tyr(1) 0.85, Val(1) 1.01. Molecular mass by FAB-MS = 1269.8 (Calc. = 1269.7). UV and CD spectra of this synthetic material (Figure 1) were consistent with previous spectroscopic analyses[6,8].

In conclusion, we have successfully used our PCOR method for the synthesis, in high yield, of TA. The Kaiser oxime resin appears to be ideally suited to the rapid assembly of cyclo-peptide

structures. The current work demonstrates the applicability of this polymer-supported cyclization procedure to the synthesis of a cyclic peptide as large as 10 residues for the first time.

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References and notes.

Symbols and abbreviations are in accordance with the recommendation of the IUPAC-IUB Commission on Biochemical Nomenclature, J. Biol. Chem. 1971, 247, 977. Amino acids are of the L configuration unless otherwise stated. TLC was carried out on silica gel G (Aldrich) with the following solvent systems: (A) $CHCl_3$/MeOH/AcOH = 6/3/1; (B) *n*-BuOH/AcOH/pyridine/H_2O = 4/1/1/2. HPLC analysis and purification were carried out using a Waters 600 Multisolvent Delivery System. Abbreviations: BOP - benzotriazol-1-yl-oxy-tris-(dimethylamino) phosphonium hexafluorophosphate; CD - circular dichroism; DCM - dichloromethane; DIEA - N,N-diisopropylethylamine; DMF - N,N-dimethylformamide; FAB-MS - fast atom bombardment mass spectrometry; TMSOTf - trimethylsilyl trifluoromethanesulfonate; Z - benzyloxycarbonyl.

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