Improved Synthesis of 4-Alkoxybenzyl Alcohol Resin

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An improved preparation of 4-(hydroxymethyl)phenoxymethyl-copoly(styrene–1% divinylbenzene) resin has been achieved by examining the kinetics of the reaction of chloromethyl resin with 4-(hydroxymethyl)phenol as a function of concentration, temperature, and base. The best product was obtained by heating chloromethyl resin with 3 equiv each of 4-(hydroxymethyl)phenol and sodium methoxide in *N,N*-dimethylacetamide for 8 h at 50 °C. Under these conditions the 1,6 elimination of 4-(hydroxymethyl)phenol to 4-quinone methide and its subsequent polymerization was greatly reduced. The colorless product contained no chlorine or nitrogen and could be esterified with a protected amino acid to the extent of 98% of the initial $ClCH_2$ sites. Trifluoroacetic acid released 93% of the amino acid and HF released 96%. The product contained less than 3% of hydroxymethyl resin or methoxymethyl resin and no more than 0.2% of resin-bound phenol. The alkoxybenzyl alcohol resin prepared by the new procedure gave an improved synthesis of the model peptide, Leu-Ala-Gly-Val, under mild conditions that avoid HF.

The success of a solid-phase peptide synthesis depends on a judicious choice of the resin support and of the protecting group strategy.[2] The alkoxybenzyl alcohol resin developed by Wang[3] primarily for the synthesis of protected peptide fragments is finding wider application in stepwise solid-phase synthesis, where mild acidic conditions for the final cleavage of the peptide from the solid support are required.[4-7] It was prepared[3] by reacting chloromethyl-copoly(styrene–1% divinylbenzene) resin beads either with 4-(hydroxymethyl)phenol and sodium methoxide for 24 h at 80 °C or with 4-hydroxybenzoic acid methyl ester, followed by reduction to the alcohol with $LiAlH_4$. We have found that the resin product obtained in our laboratory by either route or from commercial sources was not homogeneous, as determined by our inability to esterify it quantitatively under forcing conditions and to cleave the resulting ester completely with trifluoroacetic acid. In addition, the resin obtained in the former reaction was yellow colored and some chloromethyl sites remained unreacted. A standard synthesis of the test peptide, Leu-Ala-Gly-Val, gave 2–4 times higher levels of deletion peptides than with conventional chloromethyl resin or the improved PAM resin.[8] Our objective in this study was to devise more nearly optimal conditions for the synthesis of the alkoxybenzyl alcohol resin. This was achieved by examining the kinetics of the reaction of chloromethyl resin with 4-(hydroxymethyl)phenol as a function of base, concentration, and temperature.

Results and Discussion

The methoxide-catalyzed displacement of chlorine from the chloromethyl resin 1 by 4-(hydroxymethyl)phenol (**2**, 4-HMP)[9] in *N,N*-dimethylacetamide (DMA) is subject to a number of competing reactions and potential byproducts as illustrated in Figure 1. In addition to the displacement by phenoxide **3**, they include the possibility of attack by the benzyl alcohol anion **4**, by MeO^-, or by HO^-. The ability of 4-HMP to undergo 1,6 elimination to 4-quinone methide (**5**) in base or at elevated temperature is well documented,[10,11] and it was observed that 4-quinone methide formed intractable polymers under the reaction conditions. These undesirable effects during the etherification reaction will reduce the effective concentration of 4-HMP and may lead to incomplete reaction. In addition, the polymeric phenolate anions **6** may displace Cl and become resin bound. It was found that the recommended 1.3 molar equiv of 4-HMP relative to chloromethyl resin was inadequate, and 3 equiv was used for all subsequent experiments. The 4-HMP obtained from commercial sources was of very poor quality, and it was essential to recrystallize it prior to use.

Effect of Temperature. Heating 4-HMP with NaOMe in *N,N*-dimethylacetamide formed a dark, viscous liquid after 4 h at 80 °C. To avoid the polymerization side reaction, which would consume the reagent under these reaction conditions, the progress of the reaction with chloromethyl resin was followed at several temperatures by measuring the loss of Cl from the resin (Figure 2). The 4-HMP and NaOMe were both at 3 equiv relative to the $ClCH_2$ groups. At 80 °C the reaction was fast ($T_{1/2}$ 30 min), but it stopped at 90% completion. Furthermore, the resin was colored, and as shown later, the product was very heterogeneous. At 35 °C the initial rate of chloride release was slow but acceptable (half-time, 3 h); however, the competing 1,6 elimination and polymerization depleted the reagent at about 60% completion after 4 h, with no further reaction up to 24 h. At intermediate temperatures (50–65 °C) the overall rate of reaction was rapid, and at 50 °C the release of Cl was complete after 8 h. Some polymer was still formed, but the washed resin was colorless. Microscopic examination showed a normal, unaltered appearance of the beads, and they maintained their original swelling properties in organic solvents, indicating the absence of significant cross-linking side reactions. Treatment at 50 °C with only 1.3 equiv of NaOMe and 4-HMP gave a slower and less complete reaction. From this information and the data described later, it was concluded that the resulting 4-alkoxybenzyl alcohol resin was very nearly homogeneous when prepared at 50 °C with 3 equiv of each reagent.

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(9) Abbreviations: Bpoc, 2-(*p*-biphenyl)isopropyloxycarbonyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCHA, dicyclohexylamine; DIEA, diisopropylethylamine; DMA, *N,N*-dimethylacetamide; DMAP, 4-(dimethylamino)pyridine; 4-HMP, 4-(hydroxymethyl)phenol; TLC, thin-layer chromatograph y; TFA, trifluoroacetic acid.
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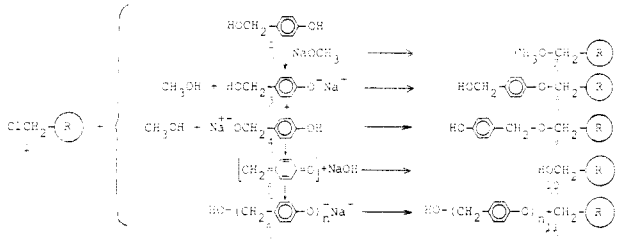

Figure 1. Potential competing reactions and byproducts in the synthesis of 4-alkoxybenzyl alcohol resin.

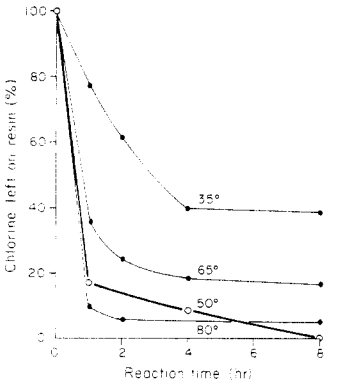

Figure 2. The influence of temperature on the $NaOCH_3$-catalyzed reaction of 4-(hydroxymethyl)phenol with chloromethyl resin. The solvent was *N,N*-dimethylacetamide (8 mL/g of resin). The 4-HMP and $NaOCH_3$ were 3 equiv each relative to the $ClCH_2$ sites. Chloride was released by quaternization with pyridine and estimated by Volhard titration.

Effect of Base. In an effort to differentially affect the competing reactions and reduce the level of formation of 4-quinone methide and its subsequent polymerization, other base catalysts were examined. Fluoride ion assisted hydrogen bonding to promote the generation of phenoxide anion was an attractive possibility. Such reactions have been reported for the etherification of phenols.[12] When 9 equiv of KF was used to promote the etherification at 50 °C, the reaction was too slow to be useful, reaching only 16% after 24 h (Figure 3). The more soluble and reactive CsF accelerated the reaction to 75% completion after 24 h, but again the rate decreased within a few hours, suggesting that HF generated in the reaction may have promoted the elimination and polymerization side reaction. Trace amounts of acid are known to accelerate such reactions. Although the HF was expected to complex with excess CsF to form $CsHF_2$, this salt may still catalyze the side reaction. Therefore, $KHCO_3$ or $CsHCO_3$ was added to its respective reaction as a buffer. The KF–$KHCO_3$ reaction remained too slow to be useful, but the CsF–$CsHCO_3$ reagent accelerated the reaction significantly ($T_{1/2}$ ~6 h). Nonetheless, the side reaction was not eliminated and the reaction leveled off at 70% completion after 24 h. $CsHCO_3$ alone catalyzed the reaction but was less effective than the mixture. Because the solubility of these alkali fluorides was not good, the much more soluble base tetraethylammonium fluoride was tested. The reaction rate was much faster than with the other fluorides, but 10% of chlorine still remained on the resin after 24 h.

Comparison of the Resin-Bound Products after the Etherification Reaction. It was important to assess the homogeneity of the resins after the reactions with 4-HMP and the various bases. The released chloride may have been displaced by $HOCH_2C_6H_4O^-$ as desired or by other nucleophiles present in the reaction mixture such as

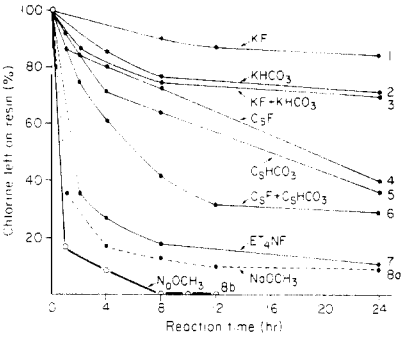

Figure 3. The influence of base on the reaction of chloromethyl resin with 4-(hydroxymethyl)phenol. The solvent was *N,N*-dimethylacetamide (8 mL/g of resin). The molar ratio of $ClCH_2$ sites/4-HMP/base was 1:3:3 except with 8a, which was 1:1:3:1.3. The reaction temperature was 50 °C except in 8a (80 °C). Chloride was released by quaternization with pyridine and estimated by Volhard titration.

$HOC_6H_4CH_2O^-$, CH_3O^-, or $HO(CH_2C_6H_4O)_n^-$. These possibilities could be distinguished by estimating the extent of esterification of all hydroxyl functions with an N^{α}-protected amino acid, followed by quantitation of the release of amino acid by acidolysis with TFA or HF or by cleavage with HO_2^-. Esterification with a Bpoc-amino acid and DCC is very effective when catalyzed by 4-(dimethylamino)pyridine. This reaction has been shown to be quantitative with primary, secondary, and tertiary alcohols and with phenols.[3,13] Amino acids esterified to resins through a 4-alkoxybenzyl ester are readily cleaved by TFA[3], whereas those esterified directly to a hydroxymethyl-polystyrene resin are resistant to TFA (~2% cleavage per hour)[2] but are released in high yield by HF. Phenolic esters are stable to either acid treatment but are cleaved by 3-h treatment with H_2O_2 in NaOH.[14] For determination of whether all of the 4-alkoxybenzyl alcohol groups are attached directly to the polystyrene or whether some of them are extended from the resin by polymerized chains of $CH_2C_6H_4O$ groups (Figure 1), the resin can be cleaved with HBr and the released $BrCH_2C_6H_4OH$ quantitated spectrophotometrically. As shown in Table I, the reaction of 1.3 equiv of 4-HMP with chloromethyl resin (0.32 mmol/g) in NaOMe at 80 °C released 90% of the chlorine, but only 39% of the initial functional sites could be esterified by Bpoc-Val (0.13 mmol/g) and only 65% of the valine could then be released by TFA, for an overall yield of 25% (0.08 mmol/g). The remaining 35% of the valine was cleaved by HF. When the esterified resin was treated with HO_2^-, only 1.5% of the Bpoc-valine was released. In the absence of 4-HMP, $NaOCH_3$ produced approximately 3% of methyl ether per hour. Finally, a sample of the resin was cleaved with HBr in HOAc, and the released $BrCH_2C_6H_4OH$, determined by its absorbance at 270 nm, was found to be 0.29 mmol/g (Table II). These data are interpreted to mean that the reaction product contained approximately 25% $HO(CH_2C_6H_4O)_nCH_2$-R (**8**) with average n = 3.6, 14% $HOCH_2$-R (**10**), 0.6% $HOC_6H_4CH_2OCH_2$-R (**9**), 50% CH_3OCH_2-R (**7**) and 10% $ClCH_2$-R (**1**). The CsF-assisted reaction released 76% of the Cl, and 55% of these sites could be esterified. Of the latter, 87% could be cleaved by TFA, indicating a 48% overall yield of 4-alkoxybenzyl alcohol resin.

The best result was achieved with 3 equiv each of 4-HMP and NaOMe in *N,N*-dimethylacetamide at 50 °C. All of the Cl was displaced, and Bpoc-Val could be es-

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Table I. Comparison of the Homogeneity of 4-Alkoxybenzyl Alcohol Resins Prepared by Reaction of Chloromethyl Resin with 4-(Hydroxymethyl)phenol and Various Bases[a]

		base							
		$NaOCH_3$			CsF	$CsHCO_3$	CsF + CsHCO	KF + $KHCO_3$	Et_4NF
		A	B	C	D	E	F	G	H
4-HMP and base	equiv[b]	3.0[c]	3.0	1.3[d]	3.0	3.0	3.0	3.0	3.0
reaction temp	°C	50	80	80	50	50	50	50	50
chloromethyl sites	mmol/g	0.000	0.031	0.025	0.078	0.116	0.093	0.237	0.034
Bpoc-Val esterified	mmol/g	0.314	0.195	0.125	0.177	0.084	0.101	0.015	0.200
	%	98	61	39	55	26	32	5	63
Val released by TFA	mmol/g	0.292	0.153	0.081	0.154	0.065	0.078	0.008	0.143
	%	93	79	65	87	77	77	53	72
Val released by HF	mmol/g	0.301		0.111			0.091		
	%	96		89			90		
Val released by H_2O_2	mmol/g	0.00055		0.0018					
	%	0.18		1.5					

[a] The initial concentration of $ClCH_2$ sites was 0.320 mmol/g. The solvent was *N,N*-dimethylacetamide (8 mL/g of resin). The reaction time was 24 h. [b] Relative to $ClCH_2$ resin. [c] These are the recommended improved conditions. [d] These are the original conditions used by Wang.[3]

Table II. Determination of the Amount of Polymerized 4-HMP on the Resin

	(hydroxymethyl)phenol units[a]		
resin	expected, mmol/g	obsd, mmol/g	av HMP units per chain[b]
unsubstituted resin	0	0	0
resin C	0.08	0.29	3.6
resin A	0.29	0.32	1.1

[a] Average of triplicate samples. [b] n = observed/expected. The expected value is equivalent to the value of valine released by TFA (Table I).

terified to the extent of 98% of the initial $ClCH_2$ sites. TFA released 93% of the valine and HF released 96%. The data indicate at least a 92% yield of 4-alkoxybenzyl alcohol sites on the resin and no more than 2% of methoxymethyl resin, 3% of hydroxymethyl resin, or 0.2% of resin-bound phenol. The data also showed that the improved resin produced 0.32 mmol of bromomethylphenol per gram, and therefore less than 10% of polymerized units could be present (n = 1.1).

To further test the properties of the 4-alkoxybenzyl alcohol resin prepared under the improved conditions, the model tetrapeptide Leu-Ala-Gly-Val was synthesized under standard solid-phase conditions, using Bpoc-protected amino acids. Cleavage with 50% TFA in dichloromethane for 2 h at 25 °C went in 87% yield. The unpurified cleavage mixture was shown chromatographically[15] to contain 99.5% of Leu-Ala-Gly-Val together with 0.3% of Leu-Gly-Val, 0.1% of Leu-Ala-Val, and none (<0.05% each) of Gly–Val, Ala-Val, Ala-Gly-Val, and Leu-Val. These results are comparable to those obtained on standard $ClCH_2$ resins of PAM resins[8] after cleavage in HF, and they show that the 4-alkoxybenzyl alcohol resin prepared under the improved conditions is satisfactory for use in stepwise solid-phase synthesis.

The primary purpose of this work was to improve the preparation of the alkoxybenzyl alcohol resin. However, the investigation was conducted in more detail in order to show the value of the solid-phase method in studies on reaction rates and mechanisms, particularly in those instances where the formation of polymeric byproducts makes the kinetics difficult to measure. In this system the procedure is simplified because the end products are covalently attached to the solid support, while the interfering byproducts are unbound and can be removed by filtation and washing. Therefore, product analysis can be achieved at intervals during the reaction without interference by soluble byproducts.

Experimental Section

Materials and Methods. Chloromethyl-copoly(styrene–1% divinylbenzene) resin (0.32 mmol of chlorine/g) was obtained from Lab Systems. Other commercial reagents were as follows: *N,N*-dimethylacetamide (Spectrograde from Eastman), 4-(dimethylamino)pyridine and sodium methoxide (Aldrich), potassium fluoride, cesium fluoride, potassium bicarbonate, and cesium bicarbonate (Alfa Chemical Co.).

4-(Hydroxymethyl)phenol (Aldrich) is not very stable. It was colored and gave two UV positive spots in the TLC system: chloroform/acetic acid/2-propanol (90:10:5), R_f 0 and 0.81. It was recrystallized from hot water as white needles in 65% yield, mp 111–112 °C; one spot, R_f 0.81 in TLC.

Bpoc-amino acid DCHA salts were from Chemalog. They were converted to free carboxylic acids with 0.5 M $KHSO_4$ adjusted to pH 3.5 with KOH.[16]

Resin hydrolysis was performed on Bpoc-valine resin samples (10–20 mg) with phenol/12 N HCl/acetic acid (1:2:1) for 24 h at 110 °C. Amino acid analyses were perfomed on the Model 121 Beckman analyzer. The amount of Bpoc-Val attached to phenolic resin was determined according to Kenner and Seeley,[14] except the time was extended to 16 h.

Synthesis of 4-Alkoxybenzyl Alcohol Resins. Chloromethyl resin (3 g, 0.96 mmol of chloromethyl sites, 1 equiv) was reacted in *N,N*-dimethylacetamide (25 mL) at 50 °C with recrystallized 4-(hydroxymethyl)phenol (0.36 g, 2.88 mmol, 3 equiv unless specified) and one of the following bases: (1) KF (0.50 g, 8.64 mmol, 9 equiv), (2) $KHCO_3$ (0.29 g, 2.88 mmol, 3 equiv), (3) KF + $KHCO_3$ (KF, 0.17 g, 2.88 mmol, 3 equiv; $KHCO_3$, 0.29 g, 2.88 mmol, 3 equiv), (4) CsF (0.44 g, 2.88 mmol, 3 equiv), (5) $CsHCO_3$ (0.56 g, 2.88 mmol, 3 equiv), (6) CsF + $CsHCO_3$ (CsF, 0.44 g, 2.88 mmol, 3 equiv; $CsHCO_3$, 0.56 g, 2.88 mmol, 3 equiv), (7) Et_4NF (0.53 g, 2.88 mmol, 3 equiv), (8a) sodium methoxide (0.069 g, 1.27 mmol, 1.3 equiv) and 4-HMP (0.15 g, 1.23 mmol, 1.3 equiv) for 24 h at 80 °C (these are the conditions described by Wang[3]), and (8b) sodium methoxide (0.16 g, 2.88 mmol, 3 equiv) at 50 °C. Samples were removed at intervals up to 72 h for analysis. Resins were washed with dioxane (4 times, 30 mL), dioxane/water (1:1) (6 times, 30 mL), dioxane (4 times, 30 mL), and methanol (3 times, 30 mL). The alkoxybenzyl alcohol resin that was synthesized by using sodium methoxide under the improved conditions 8b was colorless, but the majority of the resins were yellow.

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Determination of the Chlorine Left on the Resin. Resin samples (0.1–0.2 g) withdrawn at 1, 2, 4, 8, and 12 h and at the end of the reaction were washed, dried, and heated with pyridine (2 mL) in boiling water for 60 min. The solution with the resin was transferred to an Erlenmeyer flask with 50 mL of 20% acetic acid. A Volhard titration for chloride was carried out by addition of saturated ferric ammonium sulfate indicator (3 drops), concentrated nitric acid (5 mL), 0.1 N $AgNO_3$ (5 mL), and toluene (3 mL), followed by back-titration with 0.1 N KSCN. In the absence of the quaternization step with pyridine, no Cl was found.

Determination of the Extent of Polymerized 4-HMP on the Resin. A solution of 2 mL of 30% HBr in HOAc was added to 100 mg of the alkoxybenzyl alcohol resin (A and C) prepared by different procedures and to an unsubstituted divinylbenzene polystyrene resin control. After 1 h, the resin was filtered and washed with 1% HOAc. The filtrate was collected and diluted in 1% HOAc to 100 mL. Solid potassium bisulfite (20 mg) was added to the solution to reduce bromine to bromide. This solution absorbed strongly with maxima at 254 and 270 nm. The 270-nm absorption shifted to >330 nm when the solution was made strongly basic, indicative of conversion of the bromomethylphenol derivative to quinone methide and its products. Quantitation of bromomethylphenol was made at 270 nm ($\epsilon = 1.12 \times 10^3$ M^{-1} cm^{-1}).

Esterification of Bpoc-Val to 4-Alkoxybenzyl Alcohol Resin. 4-alkoxybenzyl alcohol resin (100 mg) was treated with Bpoc-Val (34.1 mg, 0.096 mmol), dicyclohexylcarbodiimide (19.8 mg, 0.096 mmol), and 4-(dimethylamino)pyridine (1.95 mg, 0.016 mmol) in 2.2 mL of CH_2Cl_2 in a small, screw-capped reaction vessel on a shaker for 3 h. The resin was washed with dichloromethane (6 times, 5 mL). The esterification was repeated one more time with the same excess of reagents.

Stepwise Solid-Phase Synthesis of Leucylalanylglycylvaline on 4-Alkoxybenzyl Alcohol Resin Prepared via Sodium Methoxide under Improved Conditions. The synthesis began with Bpoc-valyloxymethylphenoxymethyl resin (400 mg, 0.13 mmol). One cycle of the synthesis consisted of (1) deprotection with 0.5% TFA in CH_2Cl_2 (3 × 1 min, 1 × 20 min), (2) neutralization with 5% diisopropylethylamine in CH_2Cl_2 (3 × 2 min), (3) equilibration with Bpoc-amino acid (3 equiv, 5 min) in CH_2Cl_2, (4) coupling by addition of dicyclohexylcarbodiimide (3 equiv, 120 min). All intermediate washes were with dichloromethane. Steps 2, 3 and 4 were repeated for the second coupling. All wash and reaction volumes were 5 mL.

Protected-peptide resin (55.2 mg) was cleaved by treatment with 4 mL of 50% TFA in dichloromethane for 2 h. The resulting peptide mixture was analyzed chromatographically, before purification, for peptide composition as described previously.[15]

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Registry No. 2, 623-05-2; styrene-divinylbenzene copolymer, 9003-70-7; Bpoc-valine, 78004-68-9; leucylalanylglycylvaline, 17195-26-5; KF, 7789-23-3; $KHCO_3$, 17353-70-7; CsF, 13400-13-0; $CsHCO_3$, 15519-28-5; Et_4NF, 665-46-3; NaOMe, 124-41-4.

Synthesis of (MeAla)TANDEM, the Bis(*N*-methylalanine) Analogue of Des-*N*-tetramethyltriostin A

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[MeAla^2,MeAla^6]des-*N*-tetramethyltriostin A (**3**), an analogue of the bicyclic octadepsipeptide antibiotic triostin A (**1**), has been prepared. Analogue **3** is of interest in studies relating to the mode of binding of the triostin antibiotics to nucleic acids. The analogue contains *N*-methyl-L-alanine units in place of the two normal L-alanines and lacks the four *N*-methyl groups common to the two pair of cysteine and valine residues. Coupling *N*-methyl-L-alanine 2,2,2-trichloroethyl ester with the 2,4-dinitrophenyl ester of Z-D-serine gave dipeptide Z-D-Ser-MeAla-OTce (**6**). Depsipeptide **7** was prepared by esterification of dipeptide **6** with Boc-Val-OH by using a carbodiimide procedure catalyzed by 4-(dimethylamino)pyridine. Deprotection of **7**, followed by its coupling with Boc-Cys(Acm)-OH, gave tetradepsipeptide **8**, which, by appropriate deprotection, was converted into the respective tetradepsipeptides **9** and **10**. Fragment coupling of **9** and **10** furnished linear octadepsipeptide **11**, which upon subsequent transformation involving deprotection, cyclization, and disulfide formation gave cyclic product **13**. Removal of the *N*-[(benzyloxy)carbonyl] groups from the two D-serine residues in **13**, followed by acylation with 2-quinoxalinecarbonyl chloride, provided analogue **3**.

There is considerable interest[1] in the mode of binding of the triostin[2] and quinomycin[3] quinoxaline depsipeptide antibiotics to nucleic acids. Waring and co-workers[4] have shown these antibiotics to bind to various natural and synthetic DNA molecules by a mechanism involving bifunctional intercalation of both quinoxaline chromophores common to the antibiotics. Solution NMR conformational studies of echinomycin (quinomycin A) and of triostin A have been reported by Williams and co-workers.[5,6] A model, based on conformational energy calculations, has been proposed by Ughetto and Waring for the binding of echinomycin to DNA.[7]

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