SYNTHESIS OF α, α' -DIAMINO-1,4-BENZENEDIACETIC ACID DIMETHYL ESTER, A POTENTIAL POLYMERIZATION MONOMER

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by

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Thesis directed by Professor Douglas F. Dyckes

ABSTRACT

The synthesis of α, α' -diamino-1,4-benzenediacetic acid dimethyl ester, a potential polymerization monomer, was attempted following the method of Flack-Pedersen and Undheim. Many of the steps proceed with relatively low yields. The di-azido intermediate is especially difficult to synthesize and to convert to the protected amino acid. Our synthesis was halted at this point. Better routes are especially needed.

Several other ways to synthesize the monomer, based on the Horner-Wadsworth-Emmons (HWE) reaction and Strecker synthesis of amino acids were attempted. However, both pathways failed and we were unable to develop a convenient and inexpensive synthesis. If the monomer can be synthesized with easy and inexpensive methods, it is a potentially useful compound for polymerization because of its bis-amino acid groups. Protected forms of phenylbisglycine intermediates would also be very useful in the synthesis of cyclic peptides. The proposed polymerization reaction for phenylbisglycine was studied using phenylglycine methyl ester as a model. The model compound proved difficult to dimerize directly under mild conditions. The dimerization using classical peptide synthesis methods (protection/activation/coupling) needs to be investigated. It is probably a matter of finding the right combination of conditions and reagents.

This abstract accurately represents the content of the candidate's thesis. I recommend its publication.

Signed ______ Døuglas F. Dyckes

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1. Introduction

1.1 Goal

The essential goal of this research was to explore a pathway to synthesize α, α' -diamino-1,4-benzenediacetic acid dimethyl ester (Fig. 1.1).



Fig. 1.1 α , α' -Diamino-1,4-benzenediacetic acid dimethyl ester

The monomer could potentially be polymerized to form a new polymeric diketopiperazine (Fig. 1.2). The polymer would be of interest because diketopiperazine (DKP) is a six-membered heterocyclic compound which contains two amide bonds. The polymer may be a useful product. The amide links increase chances for biodegradability. Hydrogen bonding between the DKP's should make the chains attract each other. The repeating DKP/benzene ring structure should make the polymer fairly rigid. Oxidation/reduction of this polymer may change its properties in interesting ways.



----- : Hydrogen Bonding

Fig. 1.2 Schematic Drawing of the Polymerization

In this research, we only investigated the synthesis of the monomer. We did not polymerize these monomers. A series of different approaches to the synthesis of α, α' -diamino-1,4-benzenediacetic acid dimethyl ester are reported in this thesis.

Before the synthesis of α, α' -diamino-1,4-benzenediacetic acid dimethyl ester was attempted, a model experiment — synthesis of phenylglycine methyl ester—was carried out. Phenylglycine methyl ester could also be used in dimerization studies to find a strategy for the proposed polymerization of α, α' diamino-1,4-benzenediacetic acid dimethyl ester. Therefore, another target of this research was to explore a convenient pathway to dimerize phenylglycine methyl ester. The first step in achieving this goal was to synthesize the monomer, α -aminophenylacetic acid. Dimerization of the monomer results in a DKP-substituted with two benzene rings (Fig. 1.3). Different approaches to the synthesis of this dimer are reported in this thesis.



Fig. 1.3 The Diketopiperazine of Phenylglycine

2. Results and Discussion

2.1 Approaches to the synthesis of α,α'-diamino -1,4-benzenediacetic acid dimethyl ester

The compound α, α' -diamino-1,4-benzenediacetic acid dimethyl ester could potentially be prepared from esterification of α, α' -diamino-1,4-benzenediacetic acid. This 1,4-benzenebis(glycine) construction was very difficult and complicated. Five reaction pathways were employed in attempts to synthesize the compound:

2.1.1 First Proposed Pathway: Horner- Wadsworth -Emmons (HWE) Reaction (Fig. 2.1)

Condensation of phosphorylglycine ester (1,2) with *para*-benzoquinone in the presence of potassium *tert*-butoxide (2, 3, 4, 5) could lead to 1,4-dimethyl 1,4bis(benzyloxycarbonylamino)-2,5-cyclohexadien(idene) acetate. This product would be hydrogenated under acetic acid to yield α, α' -diamino-1,4-benzenediacetic acid dimethyl ester.

The starting material (Compound A) was synthesized after three steps (Fig. 2.2). The first step involved the nucleophilic addition of benzyl carbamate to the keto-carbonyl group of glyoxylic acid monohydrate to produce α -hydroxy-N-benzyloxycarbonylglycine. The compound was esterified with absolute methanol in the presence of concentrated H₂SO₄ to form methyl 2-benzyloxycarbonylamino-



Z: C₆H₅-CH₂-O-CO- (benzyloxycarbonyl group)

Fig. 2.1 Schematic Drawing of the First Proposed Pathway: Horner-Wadsworth-Emmons (HWE) Reaction



Methyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphinyl)-acetate

Fig. 2.2 Schematic Drawing of the Synthesis of Methyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphinyl)-acetate (Compound A)

2-methoxyacetate. The compound was first reacted with phosphorus(III) trichloride to displace methoxy group with chloride ion. In the second step, trimethyl phosphite (:P(OCH₃)₃) was added to the intermediate. The nucleophile (:P(OCH₃)₃) attacks the carbon and displaces chlorine. Electron rearrangement and elimination of one methyl group (presumably as methyl chloride) results in the formation of compound A. The assignment of the ¹H NMR (CDCl₃) spectrum (Fig. 2.3) for compound A is as follows: the methyl protons appear singlet at 3.77 ppm, the methoxy protons of the P-OCH₃ appear quartet at 3.80-3.86 ppm, the signal of -CH is a doublet of doublets resulting from splitting by two nonequivalent nearby nuclei (-NH, and P), the protons on the benzylic carbon appear singlet at 5.15 ppm, the proton on the nitrogen atom appears a broad signal at 5.65 ppm, the protons on the aromatic carbons appear as a singlet at 7.36 ppm.

A model reaction demonstrated the synthesis of an alkene (Nbenzyloxycarbonyldehydroaminoacid ester) : the phosphorylglycine ester (Compound A) was condensed with benzaldehyde in the presence of potassium *tert*butoxide at low temperature. The reaction had a high yield and pure Z-isomer of Nbenzyloxycarbonyldehydroamino acid ester was obtained from column chromatography. The ¹H NMR (CDCl₃) spectrum (Fig. 2.4) for Z-isomer produced resonances is as follows: the protons on the methoxy carbon appear as a singlet at 3.76 ppm, the protons on the benzylic carbon appear as a singlet at 5.12 ppm, the



Fig. 2.3 ¹H NMR spectrum at 200 MHz of Compound A in CDCl₃



Fig. 2.4 ¹H NMR spectrum at 200 MHz of Z-isomer of N- benzyloxycarbonyldehydroamino acid ester in CDCl₃

proton on the nitrogen atom appears as a broad signal at 6.54 ppm, the protons on the aromatic carbons and HC= appear as a multiplet at 7.24-7.62 ppm.

After treatment of phosphorylglycine ester with potassium *tert*-butoxide at low temperature and the addition of cyclohexanone, cyclohexanedione or *para*benzoquinone, the reaction was worked up by standard procedures but no alkene product could be identified with NMR from cyclohexanedione or *para*benzoquinone. The inability to react *para*-benzoquine under these conditions is probably best attributed to the stability of its conjugated pi system. The reaction did not produce the desired compound.

2.1.2 Second Proposed Pathway: Strecker Synthesis (6) (Fig. 2.5)

Reaction of terephthalaldehyde with sodium cyanide and ammonium chloride should lead to α, α' -diamino-1,4-benzenediacetonitrile. The nitrile groups could be converted to carboxylic acids after hydrolysis and then esterified.



Fig. 2.5 Schematic Drawing of the Second Proposed Pathway: Strecker Synthesis

The second proposed pathway was the Strecker synthesis. A model reaction of benzaldehyde with sodium cyanide and ammonium chloride yielded the cyanoamine. The cyanoamine product was hydrolyzed with concentrated HCl solution to convert the cyano group to carboxylic acid. The phenylglycine salt was esterified with anhydrous methanol in the presence of HCl gas to yield phenylglycine methyl ester hydrochloride. The model experiment easily produced a high yield of product.

After treatment of terephthalaldehyde with two portions of sodium cyanide and ammonium chloride under the standard procedures, there was no evidence formation of the desired biscyanoamine from the reaction. Therefore, the following reaction was used to explore the synthesis of the intermediate product, the biscyanoamine.

2.1.3 Third Proposed Pathway: (Fig. 2.6)

Reaction of terephthalaldehyde with aqueous sodium cyanide solution should yield the biscyanohydrin product (7). After conversion of the terephthalaldehyde biscyanohydrin with ammonia gas to form α, α' -diamino-1,4benzenediacetonitrile (8), the synthesis would be continued to according to the second route.

The reaction of mandelonitrile [C₆H₅-CH(CN)(OH)] in absolute ethanol and

saturated ammonia gas at 0 °C for 24 hours to yield the cyanoamine product *(8)* proceeded smoothly, providing a model for the third proposed pathway. Therefore, terephthalaldehyde was reacted with sodium cyanide to yield the biscyanohydrin product. But no evidence of biscyanohydrin product formation from the reaction could be obtained. Therefore, the following reaction was used to explore the synthesis of the biscyanohydrin intermediate.



Fig. 2.6 Schematic Drawing of the Third Proposed Pathway

2.1.4 Fourth Proposed Pathway: (Fig. 2.7)

Reaction of terephthalaldeheyde with aqueous sodium metabisulfite would yield a sodium bisbisulfite adduct $[(C_6H_4)-1,4-[CH(OH)SO_3Na]_2]$ (9,10) which can be reacted with potassium cyanide to yield the biscyanohydrin. This product would then be treated as in the third proposed reaction pathway.



Fig. 2.7 Schematic Drawing of the Fourth Proposed Reaction

The fourth proposed pathway begins with the synthesis of a bis-bisulfite intermediate en route to of the biscyanoamine product. Terephthalaldehyde was reacted with sodium bisulfite to yield the bis-bisulfite adduct. The adduct was reacted with potassium cyanide aqueous solution at 0 °C to yield the biscyanohydrin. The biscyanohydrin intermediate was then reacted in absolute ethanol and saturated ammonia gas at 0 °C for 24 hours, but the biscyanoamine product could not be obtained.

A reaction similar to the proposed pathways (second, third, and fourth) was reported in the literature (7). The reaction of α, α' -dichlorobenzene-1,4bisacetonitrile with base (ammonium thiocyanate) apparently formed the ρ -xylylene derivative which then polymerized (Fig. 2.8). In the proposed reactions, the biscyanohydrin product may react with base (ammonia) to form the biscyanohydrin anion intermediate. After electron rearrangement, elimination of one hydroxide ion (HO⁻) could form the ρ -xylylene derivative, followed by further reactions (Fig. 2.8). The desired product, the biscyanoamine, could therefore not be formed from the proposed Strecker pathways.

In fact, the monomer could not be obtained from any of the four proposed reaction sequences listed above. Therefore, another route based on a previously published method (14) was adopted to synthesize the monomer.



Fig. 2.8 Schematic Drawing of the Result of 2nd, 3rd, and 4th Proposed Pathways

2.2 Fifth Synthetic Route: (Fig. 2.9)

1,4-benzenediacetic acid (11,12) was reacted with thionyl chloride at high temperature to form the diacid chloride (13). N-acylation of lithiated 2oxazolidinone was run at -78 °C to form the bisacetamides (14). The bisacetamide was enolized by the addition of potassium hexamethyldisilazide (KHMDS) at -78 °C; enolization was effected at both α -carbons. The initially formed trisyl azide (15,16) in the substitution was converted to the azide by addition of acetic acid. The azido groups were reduced to amino groups by reduction with tin (II) chloride in aqueous dioxane and the amino compound isolated and reacted with *t*-butyloxycarbonyl anhydride-(Boc)₂O. Due to complications in isolating the desired product the synthesis was halted at this point. Synthesis would be completed by treating the Boc-protected amine with two equivalents of lithium hydroxide at ambient temperature to form the Boc-protected 1,4-benzenebis(glycine). After esterification of the 1,4-benzenebis(glycine), the monomer would be furnished.



Fig. 2.9 Schematic Drawing of the Fifth Synthetic Route

2.2.1 1,4-Phenylenediacetonitrile (Compound I)

In the synthesis of 1,4-benzenediacetyl dichloride (Compound III) involved three steps (Fig. 2.9). The first step was to synthesize 1,4-phenylenediacetonitrile (Compound I). The nitrile can be prepared from the reaction of α, α' -dichloro- ρ xylene with cyanide ion. It is an S_N 2 reaction. The first attempt to synthesize this compound followed a procedure (11) which α, α' -dichloro-p-xylene was added to a boiling solution of potassium cyanide in water and ethanol and then the hot solution was filtered immediately. However, the product still contained the starting material. After further experimentation, the reaction mixture was heated at about 50 °C for 6 hours. All of the starting material was reacted. The identity of Compound I was confirmed by ¹H and ¹³C NMR spectra, figure 2.10 and 2.11, which were compared to spectra published in *The Aldrich Library of* ¹³C and ¹H FT-NMR Spectra.

The ¹H NMR spectrum for Compound I produced resonances for the protons on the benzylic and aromatic carbons in the same proportion (1/1). These protons appear as singlets at 3.77 and 7.37 ppm. Because the benzylic protons have been shifted from downfield (4.52 ppm) in the starting material to upfield (3.77 ppm) in the product, and no signal appeared in the position (4.52 ppm), the reaction is complete. The singlet seen at 7.27 ppm corresponds to the solvent, CDCl₃. The singlet seen at 1.59 ppm was a contamination in the CDCl₃. The TMS can be seen at 0.00 ppm.



Fig. 2.10 ¹H NMR spectrum at 200 MHz of Compound I in CDCl₃



Fig. 2.11 ¹³C NMR spectrum at 200 MHz of Compound I in CDCl₃

The assignment of the ¹³C NMR spectrum for Compound I is as follows: the resonance at 21.68 ppm corresponds to the pair of benzylic carbon atoms, the resonance at 115.92 ppm corresponds to the carbons of the cyano groups, the resonance at 127.26 and 128.52 ppm correspond to the set of aromatic carbons. The solvent is seen as three large resonances at 75.48 ppm.

2.2.2 1,4-Phenylenediacetic acid (Compound II)

The second step was to synthesize 1,4-phenylenediacetic acid (Compound II). The carboxylic acid (-COOH) can be prepared from hydrolysis of the nitrile, Compound I. Compound I was refluxed in 6N HCl solution for 4 hours. The suspension mixture changed to a clear solution and then precipitated because the cyano group was converted to the amide (-CONH₂) first and then to the di-carboxylic acid. Compound II was difficult to dissolve in common organic solvents and its melting point is very high (ca. 255-258 °C). Therefore it can be dried in the oven in order to get a very dry compound for the following reaction. The identity of Compound II was confirmed by ¹H and ¹³C NMR spectra, figure 2.12 and 2.13.



Fig. 2.12 ¹H NMR spectrum at 200 MHz of Compound II in DMSO-d₆



Fig. 2.13 ¹³C NMR spectrum at 200 MHz of Compound II in DMSO-d₆

The ¹H NMR spectrum for Compound II produced resonances for the protons on the benzylic and aromatic carbons in the same proportion. These protons appear as singlets at 3.54 and 7.19 ppm, integrating to four protons. There is a broad peak between 12.00 and 12.60 ppm, integrating to two protons, from carboxylic acid. The singlet seen at 2.51 ppm corresponds to the solvent, DMSO-d₆. The singlet seen at 3.39 ppm was a contamination in the DMSO-d₆. The TMS can be seen at 0.00 ppm.

The assignment of the ¹³C NMR spectrum for Compound II is as follows: the resonance of the benzylic carbons is combined with the resonance of the solvent between 37.1 and 39.6 ppm, the resonances at 128.20 and 132.25 ppm correspond to the set of aromatic carbons. The resonance at 171.73 ppm is identified as the carbonyl carbons of the carboxyl group.

2.2.3 1,4-Phenylenediacetyl dichloride (Compound III)

Compound III was synthesized by converting Compound II into its acyl chloride. The first attempt to synthesize Compound III followed a procedure in which 1,4-phenylenediacetic acid, HMDS and TMS-Cl in dry dichloroethane were refluxed overnight and then reacted with oxalyl chloride. A straw yellow, sticky mixture was formed. As judged from its NMR spectrum, many impurities contaminated the product and just small amount of Compound III had formed. Compound III is very unstable especially in the presence of moisture. If there is water in the reaction, Compound III easily returns to the starting material. After further experiments, a procedure was found in which the starting material suspended in thionyl chloride and dry dichloromethane (dried by MgSO₄) was refluxed in an oil bath until the suspension mixture changed clear. After removing the solvent and the excess thionyl chloride, petroleum ether was added to dissolve the residue. After filtering the petroleum ether solution, the filtrate crystallized upon storage in the freezer. The colorless crystals are very pure as judged by ¹H NMR, and had to be used immediately. The identity of Compound III was confirmed by ¹H, ¹³C NMR spectra and melting point.

The ¹H NMR spectrum for Compound III (Fig. 2.14) produced resonances for the protons on the benzylic and aromatic carbons in the same proportion. These protons appear as singlets at 4.16 and 7.30 ppm. The singlet seen at 1.59 ppm was a contamination in the CDCl₃. The TMS can be seen at 0.00 ppm.


The assignment of the ¹³C NMR spectrum for Compound III (Fig. 2.15) is as follows: the resonance at 51.02 ppm corresponds to the benzylic carbons, the resonances at 128.64 and 129.82 ppm correspond to the set of aromatic carbons. The resonance at 170.20 ppm is identified as the carbonyl carbons.



Fig. 2.15 ¹³C NMR spectrum at 200 MHz of Compound III in CDCl₃

2.2.4 (S,S)-β,β'-bis(2-oxo-3-oxazolidinyl)-1,4diethylbenzene (Compound IV)

There are two steps to yield Compound IV (Fig. 2.16). 2-Oxazolidinone was reacted with butyllithium under nitrogen at -78 °C in the presence of triphenylmethane (indicator) to form the anion of 2-oxazolidinone. Excess butyllithium reacts with triphenylmethane to form its deprotonated anion. The reaction mixture changed from white to pale orange because the deprotonated anion formed. If the pale orange persisted, the lithiated 2-oxazolidinone was completely formed. Compound III was then added dropwise to the reaction mixture to yield the diacetamide (Compound IV). At first, an attempt was made to purify the crude compound with column chromatography, eluting with hexanes: ethyl acetate (1: 2, by volume). But the crude compound did not dissolve in the eluent. After the crude compound was suspended and stirred in hexanes: ethyl acetate (1:2) for 20 minutes, the insoluble portion was collected by filtration and dried with oil pump. This residue turned out to be the desired compound in a very pure form.

The ¹H NMR spectrum for Compound IV (Fig. 2.17) is as follows: there is a triplet upfield between 3.96 and 4.06 ppm integrating to 4 protons which corresponds to the $-CH_2N$ protons, the resonance at 4.27 ppm corresponds to the protons on the benzylic carbon, there is a triplet downfield between 4.37 and 4.50 ppm integrating to 4 protons which corresponds to the $-CH_2O$ protons, the singlet seen at 7.28 ppm represents the aromatic protons integrating to 4 protons. All four signals are in the same proportion.



1,4-benzenediacetyl dichloride



Fig. 2.16 Schematic Drawing of the Synthesis of Compound IV



Fig. 2.17 ¹H NMR spectrum at 200 MHz of Compound IV in CDCl₃

The assignment of the ¹³C NMR spectrum for Compound IV (Fig. 2.18) is as follows: the resonance at 39.11 ppm corresponds to the pair of benzylic carbons, the resonance at 41.10 ppm corresponds to the $-CH_2N$ carbons, the resonance at 60.41 ppm corresponds to the $-CH_2O$ carbons, the resonances at 128.43 and 130.98 ppm correspond to the set of aromatic carbons. The resonances at 151.99 (-N-C=O) and 169.76 ppm (CH₂C=O) are identified as carbonyl carbons.



Fig. 2.18 ¹³C NMR spectrum at 200 MHz of Compound IV in CDCl₃

2.2.5 2,4,6-Triisopropylbenzenesulfonyl Azide (Compound V)

The reaction to form Compound V is an S_N1 reaction. Azide ion replaces the chloro-group and forms 2,4,6-triisopropylbenzenesulfonyl azide (Fig. 2.19). ... The compound is unstable at high temperature. Trisyl azide is potentially explosive. After evaporation to remove the solvent, the oily compound was stored in the freezer to solidify. It was prepared fresh each time for synthesis of Compound VI and used without further purification. The identity of Compound V was confirmed by melting point, ¹H NMR, and IR spectra.

The observed melting point range, 38-39 °C, agreed with literature values (41-43 °C). The ¹H NMR spectrum (Fig. 2.20) for Compound V appears as follows: there is a multiplet between 1.21 and 1.35 ppm integrating to 18 protons which corresponds to the protons of methyl carbons. The methyne protons of the *ortho*-and *para*-isopropyl groups appear as two multiplets. The upfield multiplet between 2.83 and 3.05 ppm integrating to one proton, represents the proton of the *para* substituent. The *ortho* substituent protons correlate to a multiplet downfield between 3.97 and 4.18 ppm integrating to two protons, the singlet seen at 7.23 ppm is the aromatic protons. The azide group is seen at 2120 cm⁻¹ in the IR spectrum (Fig. 2.21).



.....

Fig. 2.19 Schematic Drawing of the Synthesis of Compound V



Fig. 2.20 ¹H NMR spectrum at 200 MHz of Compound V in CDCl₃



Fig. 2.21 IR spectrum of Compound V in Nujol

2.2.6 α,α'-Diazido-β,β'-bis(2-oxo-3-oxazolidinyl)-β,β' -dioxo-1,4-diethylbenzene(Compound VI)

There are several difficulties with synthesis of Compound VI. The biggest one is solubility. Compound IV can not be dissolved in THF. In the first attempt to synthesize Compound VI, the suspension of Compound IV was added to the reaction mixture with a syringe. The suspension was difficult to inject to the solution. Therefore, just a small amount of starting material was introduced to the mixture and a very low yield formed, even though washing with more solvent (THF) was used. In later reactions, the suspension was added to the solution via a cannula. In this study, all of Compound IV was introduced in the reaction mixture as a suspension.

The second problem is the reaction time. The first attempt to synthesize the compound followed the procedure of Falck-Pedersen and Undheim. (14) However, the result was not good. There was more starting material left than product. The reaction time was increased from 30 minutes to two hours.

The third problem is moisture. The reaction is very moisture sensitive. Because the solvent (THF) easily absorbs water, it needs to be dried carefully before use.

Actually, the reaction is a very complicated and difficult. There are three steps (Fig. 2.22) in the reaction as well as five steps to get the starting materials (Compound VI) (Fig. 2.9). The first step was to add Compound IV suspended in THF to potassium bis(trimethylsilyl) amide solution at -78 °C, to form the potassium enolate. Enolization was effected at both α -(benzylic)carbons. Next, freshly formed trisyl azide in dry THF was added to the potassium enolate solution at -78 °C. The last step was quenching by addition of acetic acid at 30 °C. The crude product still contained starting material. Therefore, it needed to be purified. The crude product was hard to dissolve in ethyl acetate. After column chromatography, the yield of pure product is very low (0.3 %). In order to get more product for the following reaction, the purification of Compound VI was skipped.

The ¹H NMR spectrum for Compound VI (Fig. 2.23) is as follows: there is a triplet upfield between 3.96 and 4.07 ppm integrating to 4 protons which corresponds to the –CH₂N protons, the resonance at 4.27 ppm corresponds to the protons on the benzylic carbon (Compound IV), there is a triplet downfield between 4.38 and 4.46 ppm integrating to 4 protons which corresponds to the –CH₂O protons, the singlet seen at 6.22 ppm corresponds to the protons on the benzylic azido carbon (Compound VI), the singlet seen at 7.28 ppm presents the aromatic protons integrating to 4 protons. From the spectrum, there was still much (about half) starting material (Compound IV) in the crude product.



Fig. 2.22 Schematic Drawing of the Synthesis of Compound VI



2.2.7 α,α'-Bis(t-butoxycarbonylamino)-β,β'-bis(2oxo-3- oxazolidinyl)-β,β'-dioxo-1,4-diethylbenzene (Compound VII)

The first synthesis was a modification of the procedures described by Falck-Pedersen and Undheim. (14) In this study, the azido groups in the Compound VI are reduced to amino groups by the reaction with tin (II) chloride in aqueous dioxane. Then the amino compound is protected by reaction with *t*-butyloxycarbonyl anhydride ((Boc)₂O). In our synthesis, before the reaction with (Boc)₂O, TLC analysis indicated a ninhydrin-positive (orange) component with an $R_{t}=0$ (hexanes:ethyl acetate 2:1). After reacting with (Boc)₂O, the orange spot disappeared. This could indicate acylation of an amine. The reaction mixture was extracted with ethyl acetate, washed with aqueous sodium bicarbonate, dried over sodium sulfate and then evaporated. The crude product was examined with NMR. No signal appeared at 5.50 ppm (CHNH.Boc). Either Compound VI was not reduced to amino group by tin chloride in aqueous dioxane, or something went wrong in the protection step.

The second reaction was carried out without (Boc)₂O protection of the amino group. At first, the orange spot showed on the TLC plate. After extraction with ethyl acetate, no ninhydrin-positive spot appeared in the organic and aqueous layers. ¹H NMR analysis indicated, no signal corresponding to CHNH₂. But, there were signals at 4.27 ppm (Compound IV) and 6.13 ppm (Compound VI, CHN₃).

Therefore, the reductive reaction for Compound VI was not successful in these conditions.

Compound VII may need more reaction time. However, the starting material (Compound VI) was hard to obtain. This line of research was stopped in this step.

2.3 Exploration of the Dimerization of Phenylglycine methyl ester (Fig. 2.24)

Formation of the dimer, 3,6-Diphenyl 2,5-piperazinedione, involves the combining of two units of phenylglycine methyl ester, one to the other, in two peptide linkages. The reaction involves the loss of two molecules of methanol (17).

The product has found uses in agriculture. Leaves and stems of soybean plants which were treated with this compound, can increase their weight more than those of untreated plants. (18) It was prepared as a plant growth stimulator, strongly stimulating foliage growth in cabbage and lettuce. (19) The dimer was described as a microbicide, especially for Erwinia aroideae. (20)

The compound has been prepared by heating phenylglycine methyl ester at 200 °C (19). However, we wanted to find milder conditions to synthesize the dimer. For example, phenylglycine methyl ester hydrochloride could be treated with triethylamine and stirred in an aprotic solvent at room temperature to yield the dimer. Also, a catalyst such as dimethylamino pyridine (DMAP) could be used to accelerate the reaction (Fig. 2.24). A variety of different conditions were explored.



Fig. 2.24 Schematic Drawing of the Dimer Reaction

2.3.1 α-Amino-phenylacetic acid (Compound VIII)

In this part of the research, α -amino-phenylacetic acid (phenylglycine) was prepared by Strecker synthesis. (6) Benzaldehyde was reacted with sodium cyanide and ammonium chloride to yield aminophenylacetonitrile. Phenylglycine was prepared by hydrolysis of the aminophenylacetonitrile with concentrated hydrochloric acid. The bright yellow crude product was almost dried and then purified as described in section 3.3.12. After purification, pale yellow lustrous platelets were obtained (30.3 % yield). In the first attempt, the crude product was purified without first drying, and only an 18.5 % yield was obtained. The identity of Compound VIII was confirmed by its ¹H spectrum (Fig. 2.25), which was compared to one published in *The Aldrich Library of* ¹³C and ¹H FT-NMR Spectra. The assignment of the ¹H NMR spectrum for Compound VIII is as follows: the singlet seen at 4.94 ppm corresponds to the benzylic proton, the broad singlet seen at 7.23-7.25 ppm represents the aromatic protons. The NMR solvent was D₂O with small amount of DC1. Therefore, the protons of NH and COOH are not seen in the spectrum.



Fig. 2.25 ¹H NMR spectrum at 200 MHz of Compound VIII in D₂O and DCl

2.3.2 α-Amino-phenylacetic acid methyl ester. HCl (Compound IX)

 α -Amino-phenylacetic acid was esterified with anhydrous methanol in the presence of hydrochloric acid gas to yield phenylglycine methyl ester hydrochloride. The identity of Compound IX was confirmed by ¹H NMR spectroscopy and comparison to spectra published in *The Aldrich Library of* ¹³C and ¹H FT-NMR *Spectra*. The assignment of the ¹H NMR spectrum (Fig. 2.26) for Compound IX is as follows: the singlet seen at 3.71 ppm is the methyl protons, the singlet seen at 5.27 ppm is the benzylic proton, the multiplet seen at 7.44-7.54 ppm was the aromatic protons, the broad signal seen at 9.20 ppm represents the protonated amine group. The NMR solvent is DMSO-d₆. The synthesis of phenylglycine methyl ester hydrochloride was easy and gave a high yield.



Fig. 2.26 ¹H NMR spectrum at 200 MHz of Compound IX in DMSO-d₆

2.3.3 3,6-Diphenyl 2,5-diketopiperazine (Compound X)

There were several ways found in the literature to synthesize phenylglycine anhydride (3,6-diphenyl 2,5-diketopiperazine). According to these papers (17-20, 22), they used high temperature or high temperature and high pressure to condense phenylglycine methyl ester to its anhydride. However, we wished to synthesize the compound using milder conditions. From the synthesis of glycine anhydride, (17) triethylamine was added to glycine ethyl ester hydrochloride in water to yield glycine ethyl ester. The reaction mixture was stirred at room temperature for two days to form glycine anhydride. Therefore, phenylglycine methyl ester hydrochloride was treated in the same way to synthesize its anhydride. Because phenylglycine methyl ester did not dissolve in water, some methanol was added to the reaction mixture. After stirring at room temperature for two days, the white fine crystals were collected and then analyzed by ¹H NMR. The ¹H NMR spectrum was similar to the spectrum of phenylglycine. From the literature, (22) the protons (Ph-CH) showed at 5.62 ppm on ¹H NMR spectrum. The only apparent reaction was hydrolysis of the starting material. Though the solvent was changed to aprotic solvent (DMSO and DMF) and a catalyst (DMAP) was used to accelerate the reaction, the monomer could not readily be dimerized.

Application of the methods used for glycine dimerization when used for the synthesis of phenylglyine dimer were unsuccessful. The difference in these two d_{1} starting materials is the α -phenyl group. Presumably because of crowding, phenylglycine yields a dimer with greater steric energy than the dimer of glycine.

2.4 Conclusions

I: Model Compound: 3,6-diphenyl diketopiperazine

Investigation of the dimerization of phenylglycine using classical peptide synthesis method (protection/activation/coupling) instead of the milder conditions tried here, should be pursued. The starting material-phenylglycine methyl ester can be easily synthesized in high yield. Therefore, it remains a good model for continuing this research.

II: Target Compound: α, α' -Diamino-1,4-benzenediacetic acid dimethyl ester

The proposed pathways for synthesizing the target compound without protecting groups all failed. Therefore, the compound was synthesized following the route of Falck-Pedersen and Undheim (14). Because the research was not concerned with the chirality of the product, the protecting group 2-oxazolidinone was used instead of (S)-4-benzyl-2-oxazolidinone. The Compound IV is easy to purify and in good yield, but the following reaction sequences need to be optimized and simplified. It is a long and difficult synthetic route. The biggest problem is

formation of the di-azido product (Compound VI). The reaction is very hard to run and the yield is very low. Because this intermediate (Compound VI) is difficult to produce, the following reactions could not be continued. If Compound VI can be easily synthesized, the following products which are protected amino acids would be very useful for peptide synthesis.

2.5 Further Studies

The target compound is potentially useful for polymer research. If it can be synthesized in a convenient way, it is interesting to explore the new potential polymer. However, after several attempts, the target compound still could not be synthesized in this research. The further studies to explore a more convenient synthetic route will be needed in the future.

3. Experimental

3.1 Reagents

Benzyl carbamate (98 %), glyoxylic acid monohydrate (98 %), phosphorus trichloride (98 %), benzaldehyde (99+ %), trimethyl phosphite (99+ %), potassium *tert*-butoxide (95 %), cyclohexanone (99+%), 1,4-cyclohexanedione (98 %), 1,4benzoquinone (98 %), α,α' -dichloro- ρ -xylene (98 %), 2- oxazolidone, butyllithium (1.6 M solution in hexanes), potassium bis(trimethylsilyl)amide (0.5 M solution in toluene), 2,4,6-triisopropylbenzenesulfonyl chloride (97%) were purchased from Aldrich. Sodium azide (99 %) was purchased from Sigma. Triphenylmethane was purchased from Mattheson Colleman & Bell. Stannous Chloride and triethylamine (98 %) were purchased from J.T. Baker. Sodium cyanide (95 %) was purchased from EM Science. Potassium cyanide (96 %) was purchased from Mallinckrodt.

3.2 General Methods

3.2.1 Thin Layer Chromatography

Thin layer chromatography (TLC) was performed using aluminum plates pre-coated with 0.2 mm layer of silica gel 60 (F_{254}) (EM Science). Samples were spotted on the plates using a 1 μ L micropipet and the chromatograms were developed in certain solvent system which was described in the synthetic procedure section. The identification of the components was determined by one of the following methods:

System A: Plate was sprayed with a solution containing 2 % ninhydrin in acetone, and then heated for 10 minutes at 110 °C.

System B: plate was observed under ultraviolet light (254 nm) and then immersed in silica gel saturated with iodine for a brief period.

3.2.2 Column Chromatography

Column chromatography was used to purify crude products. The stationary phase of the column was 230 -240 mesh (60 Å) silica gel. 150 mL of silica gel was packed in a column by the slurry method and thoroughly washed with the eluent. The crude product in eluent was applied to the top of the column. After forerunning about 60 –80 mL of eluent, many fractions (8 mL /each) were collected. TLC monitoring indicated the desired product in certain fractions. These fractions were pooled together and the solvent was removed with a rotary evaporator.

3.2.3 Nuclear Magnetic Resonance Spectroscopy

Nuclear magnetic resonance analyses were performed using a Varian 200 MHz spectrometer. Chemical shifts, δ , are reported in ppm. Solutions were prepared in CDCl₃, acetone-d₆ or in DMSO-d₆ containing TMS ($\delta = 0.00$) as internal reference or in D₂O.

3.2.4 Melting Points

Melting points were determined by using a Mel-temp Laboratory Devices melting point apparatus on a solid sample in a thin-walled capillary tube (1 mm × 100 mm). All melting points reported are given without correction.

3.2.5 Infrared (IR) Spectroscopy

IR spectra were recorded on Nicolet Magna IR 550 Spectrophotometer. All spectra were obtained at room temperature using KCl pellets.

3.2.6 Drying of the organic solvents

The drying was carried out as follows: Al_2O_3 and molecular sieves (4 Å) were dried in the oven at 180 °C for two hours before use. The molecular sieves were put in the flask which is connected with nitrogen to remove the air within the flask. THF was passed through the dried Al_2O_3 in a column. The forerun was discard and then the following solvent was collected. The solvent was stored over the dried molecular sieves.

3.3 Synthetic Procedures

3.3.1 First Proposed Pathway

The starting material, methyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphinyl)-acetate (phosphorylglycine ester), was synthesized using the procedures described by Zoller and Ben-Ishai (1) and Schmidt, Lieberknecht, and Wild (2). The strategy for synthesizing the starting material (Compound A) is schematically drawn in Fig. 2.2.

α-Hydroxy-N-benzyloxycarbonylglycine: A mixture of benzyl carbamate (7.25 g, 48mmol) and glyoxylic acid monohydrate (4.86 g, 52 mmol) in anhydrous ether (48 mL) was stirred overnight. The white emulsion was cooled in an ice bath to solidify and then filtered. The crystalline product was washed with dry ether and used without further purification in the following step. Yield: 10.75 g (99.5 %), m.p.: 176-178 °C (lit. m.p.: 196-198 °C dec) (1). ¹H NMR (acetone-d₆), δ (ppm) 5.08 (s, 2H, OCH₂), 5.40-5.42 (t, 1H, CH), 7.32 (s, 5H, aromatics).

benzyloxycarbonylglycine (11.09 g, 49 mmol) in cold absolute methanol (115 mL) cooled in an ice bath was added concentrated H_2SO_4 (96.4%, 1.6 mL). The reaction mixture was stirred for 2 days at room temperature and then poured into ice-cold saturated sodium bicarbonate solution (100 mL). The organic material was extracted with ethyl acetate (3 × 100 mL). The combined organic layers were dried with MgSO₄, filtered and then the solvent was evaporated. The crude oily material

Methyl 2-benzyloxycarbonylamino-2-methoxyacetate: To α-hydroxy-N-

was crystallized under petroleum ether (30 mL) in the freezer. The white crystalline product was filtered and then purified with column chromatography.

The crude product was dissolved in eluent (chloroform/methanol: 8/2) and applied to the top of the standard silica gel column. After passing about 70 mL of eluent, 25 fractions (8mL/each) were collected. TLC monitoring indicated the desired product in fractions 5-17. These fractions were pooled together and the solvent was removed by rotary evaporator. The oily residue was then dried under nitrogen gas. Finally, the purified product solidified as light yellow crystals. Yield: 11.6 g (92.9 %), m.p.: 71-73 °C (lit. m.p.: 76-78 °C dec) (1). ¹H NMR (CDCl₃), δ (ppm) 3.44 (s, 3H, OCH₃), 3.82 (s, 3H, COOCH₃), 5.18 (s, 2H, CH₂Ph), 5.36-5.40 (d, 1H, CH), 5.80-5.90 (br d, 1H, NH), 7.39 (s, 5H, aromatics),

Methyl 2-benzyloxycarbonylamino-2-(dimethoxyphosphinyl)-acetate

(Compound A): Methyl 2-benzyloxycarbonylamino-2-methoxyacetate (11.5 g, 46 mmol) was dissolved in toluene (46 mL) at about 70 °C and phosphorus (III) chloride (4.9 mL, 46 mmol) was added to the solution. The colorless reaction mixture was stirred at about 70 °C overnight after which trimethyl phosphite (4.5 mL, 46mmol) was added dropwise. Stirring was continued for a further 2 hours at 70 °C. The toluene was evaporated with a rotary evaporator and the residual product (a yellow emulsion) was dissolved in ethyl acetate (50 mL). The mixture was washed with sodium bicarbonate solution (3 × 50 mL), dried over sodium sulfate, filtered and then evaporated. The residual product was mixed with hexanes

(40 mL) with vigorous stirring to precipitate product. The product solidified as white fine crystals. Yield: 11.4 g (75.6 %), m.p.: 76-77 °C (lit. m.p.: 80 °C dec) (2). ¹H NMR (CDCl₃), δ (ppm) 3.77 (s, 3H, COOCH₃), 3.80-3.86 (m, 6H, P-OCH₃) 4.86-5.02 (dd, 1H, CH), 5.15 (s, 2H, CH₂Ph), 5.65 (br d, 1H, NH), 7.36 (s, 5H, aromatics).

Model Reaction and Attempted Synthesis of 1,4-Dimethyl 1,4-

bis(benzyloxycarbonylamino)-2,5-cyclohexadien(idene) acetate : Potassium *tert*-butoxide (0.34 g, 3 mmol) was cooled in a dry ice/acetone bath and then dichloromethane (0.8 mL) was added dropwise to the cooled base under nitrogen gas. Compound A (0.99 g, 3 mmol) in dichloromethane (4.6 mL) was added dropwise to the cooled mixture. Once the potassium tert-butoxide was totally dissolved, benzaldehyde (0.31 mL, 3 mmol) in dichloromethane (0.8 mL) was added dropwise to the reaction mixture. The solution was stirred at -70 °C for another 30 minutes and then for 2 hours at room temperature. In the meantime, a yellow syrup formed. The mixture was concentrated with a rotary evaporator and the residue was redissolved in ethyl acetate (25 mL). The solution was washed with water (15 mL) and ammonium chloride (10 mL). The organic layer was dried over sodium sulfate, filtered, and evaporated. The crude product was yellow syrup. Yield: 0.90 g (97 %). The crude product was dissolved in eluent (hexanes/ethyl acetate: 8/2) and applied to the top of the standard silica gel column. After passing about 125 mL of eluent, 60 fractions (8mL/each) were collected. TLC monitoring indicated the desired product in fractions 13-27 ($R_f = 0.47$) and 30-57 ($R_f = 0.33$). These fractions were pooled together and the solvent was removed with a rotary evaporator. The first product (E-isomer) was a light yellow syrup, 0.046 g (4.9 % yield). The second product (Z-isomer) was also a light yellow syrup, 0.758 g (81.5 % yield). ¹H NMR (CDCl₃) for the Z-isomer, δ (ppm) 3.76 (s, 3H, COOCH₃), 5.12 (s, 2H, CH₂Ph), 6.54 (br s, 1H, NH), 7.24-7.62 (m, 11H, aromatics and CH).

The same conditions were used in reaction of compound A with cyclohexanone, 1,4-cyclohexandione, and *para*-benzoquinone. For cyclohexanone, the reaction solvent was changed to CH₃CN and the reaction time at room temperature was prolonged to two days. There was some evidence of the desired product appearing in the NMR spectrum of the crude solid. The ¹H NMR spectrum at room temperature shows in part several broadened signals indicating a conformational equilibrium just above the coalesence. The ¹³C NMR spectrum was compared to the literature and indicated that the desired product was probably present. The ¹³C NMR spectrum although contaminated with several by-products also showed peaks corresponding to those published by Schmidt et.al, *(3)*. For 1,4-cyclohexandione and *para*-benzoquinone, the reaction solvent was also changed to CH₃CN and the reaction time at room temperature was prolonged to four days, the crude product was fractionated with column chromatography, but no evidence of either desired product was found from NMR spectroscopy of the chromatographic fractions.

3.3.2 Second Proposed Pathway

The model reaction for this method was the preparation of phenylglycine (Compound VIII), whose synthesis is described in Section 3.3.12. That product was easily obtained in good yield. The synthesis in the second pathway uses the procedures described for compound VIII.

Sodium cyanide (95%, 5.16 g, 0.10 mol) was dissolved in water (20 mL) and then ammonium chloride (5.9 g, 0.11 mol) was added to the aqueous solution. Terephthalaldehyde (6.71 g, 0.05 mol) in methanol (100 mL) was added to the above aqueous solution. The reaction mixture changed from white to yellow and the temperature rose. The reaction was stirred at room temperature overnight. The mixture was diluted with water (100 mL) and then extracted with ether (100 mL). The organic layer was shaken with concentrated HCl solution, one 30 mL portion and two 15 mL portions. The combined acid extracts were refluxed for two hours. The acidic mixture was neutralized with ammonium hydroxide (28 mL). Some dark green crystals formed. The crystals were filtered, washed with water (3×20 mL), and dried. After examining with NMR, no evidence was found of the desired product. Therefore, the following proposed pathways were to explore the synthesis of the intermediate compound, terephthalaldehyde biscyanoamine.

3.3.3 Third Proposed Pathway

The intermediate compound, terephthalaldehyde biscyanoamine, was synthesized according to modified procedures described by Minovici and Bente (8). The first step was to synthesize terephthalaldehyde biscyanohydrin (7). Terephthalaldehyde (1.34 g, 10 mmol) was added to aqueous sodium cyanide (50 mmol) and stirred at room temperature for two hours. A precipitate formed. The reaction mixture was filtered and the collected product was washed with ether. The residual solid was dissolved in absolute ethanol (5 mL) and ammonia gas was passed through the solution for about 30 minutes in an ice bath. The reaction flask was then sealed and stirred in the refrigerator for 24 hours. The reaction mixture was opened after 24 hours. No crystals formed from the mixture. TLC of the mixture showed only starting material present. Therefore, more ammonia gas was added to the cooled mixture and the reaction was stirred in the refrigerator for 4 days. After a long time in cold storage, some crystals formed from the reaction mixture. The crystals were collected and then dried. Actually, after filtration, some crystals disappeared and very little product was left. TLC was used to check the filtrate and starting material, but there was no new spot appearing. The filtrate and starting material had the same signals on the TLC plate. The solid was examined with NMR but no evidence was found of the desired product.

3.3.4 Fourth Proposed Pathway

This proposed pathway was to explore the synthesis of the intermediate compound, terephthalaldehyde biscyanohydrin. terephthalaldehyde biscyanoamine, which was synthesized according to modified procedures described by Buck (9) and Kratzer, et. Al,.(10) Sodium metabisulfite (4.75 g, 0.025 mol) was dissolved in water (15 mL). Once sodium metabisulfite had totally dissolved in water, terephthalaldehyde (1.34 g, 0.01 mol) was slowly added to the solution. And the reaction mixture was heated to 50 °C, then cooled to 0 °C in an ice bath. Sodium cyanide (2.58 g, 0.05 mol) in water was added dropwise to the stirred cooled mixture. The reaction mixture was still stirred in an ice bath. Ammonia gas was added to the stirred reaction mixture for 20 minutes. After ammonia gas was stopped, the reaction mixture was still stirred in an ice bath overnight. The mixture changed color from white to pale yellow. More crystals formed. These crystals was collected and then dried in the desiccator. The solid was examined with NMR but no evidence was found of the desired product.
3.3.5 Synthesis of 1,4-phenylenediacetonitrile (Compound I)

Compound I was synthesized according to modified procedures described by Titley (11), and Marvel and Kraiman (12). Potassium cyanide (10.1 g, 96%, 0.149 mol) was dissolved in water (22 mL) and ethanol (95%, 75 mL) was added to the aqueous solution. The mixture was heated in a water bath at about 50 °C. α , α' -Dichloro-*p*-xylene (11.8 g, 98 %, 0.066 mol) was added to the stirred mixture. The suspension was stirred for about 6 hours in a ca. 50 °C water bath. The hot clear orange solution was cooled in an ice bath. After cooling, the reaction mixture formed more crystals. The precipitate was collected on a fritted funnel, washed with water (200 mL) and water/ethanol (1:1, 150 mL), and dried in a desiccator over CaSO₄. Yield: 7.35 g (71.2 %), m.p.: 93 °C (lit. m.p.: 96 °C) (11). ¹H NMR (CDCl₃), δ (ppm) 3.77 (s, 4H CH₂), 7.37 (s, 4H, aromatics), ¹³C NMR (CDCl₃), δ (ppm) 21.68 (CH₂), 115.92(C=N), 127.26, 128.52 (aromatics).

3.3.6 Synthesis of 1,4-phenylenediacetic acid (Compound II)

Compound II was synthesized according to modified procedures described by Marvel and Kraiman,(*12*) and Wenner.(*13*) Compound I (7.32 g, 0.047 mol) was suspended in 6 N HCl solution (115 mL). The mixture was refluxed for 4 hours. As the reaction proceeded, the suspension turned to a clear solution and then to suspension. After cooling, the white precipitate was collected on a fritted funnel, washed with water (200 mL) and then with H₂O-EtOH (1:1, 200 mL). The white fine powder was dried in the oven. Yield: 6.84 g (75.2 %), m.p. 255-258 °C (lit.: 249-251 °C). ¹H NMR (DMSO-d₆), δ (ppm) 3.60 (s, 4H, CH₂), 7.20 (s, 4H, aromatics), 12.20-12.40 (br s, 2H, COOH). ¹³C NMR (CDCl₃), δ (ppm) 39.61 (CH₂), 128.20, 132.25 (aromatics), 171.73 (C=O).

3.3.7 Synthesis of 1,4-phenylenediacetyl dichloride (Compound III)

Compound III was synthesized according to the procedure described by Marvel and Draiman,(*13*) and Beyermann et. al, (*21*). Compound II (2 g, 0.01 mol) was suspended in dry dichloromethane (10 mL) and thionyl chloride (4 mL). The suspension was heated in an oil bath until the reaction mixture was clear. The reaction mixture was evaporated to remove the solvent and dichloromethane was added to the residue and reevaporated (3×40 mL) to remove the excess thionyl chloride. Petroleum ether was used to dissolve added the pale yellow residue and then filtered. The clear filtrate was stored in the freezer. Colorless fine crystals formed from the clear solution. The fine crystals were collected on a fritted funnel. Yield: 1.17 g (50.0 %), mp 61-62 °C (lit.: 66-66.5 °C). ¹H NMR (CDCl₃), δ (ppm) 4.16 (s, 4H, CH₂), 7.30 (s, 4H, aromatics). ¹³C NMR (CDCl₃), δ (ppm) 51.02 (CH₂), 128.64, 129.82 (Ph), 170.20 (C=O).

3.3.8 Synthesis of (S,S)-β,β'-bis(2-oxo-3-oxazolidinyl) -1,4-diethylbenzene (Compound IV)

Compound IV was synthesized according to the procedure described by Falck-Pedersen and Undheim (14). 1.6 M n-BuLi in cyclohexane (5.6 mL, 8.96 mmol) was added dropwise to a solution of 2-oxazolidinone (757 mg, 98 %, 8.69 mmol) and triphenylmethane (3 mg) in dry THF (30 mL) under nitrogen in a dry ice bath. The reaction mixture was stirred at -78 °C until a pale orange color persisted. Compound III (1.01 g, 4.33 mmol) in dry THF (10 mL) was added dropwise to the reaction mixture and stirred for 10 minutes. The mixture was warmed to about 25 °C, saturated aqueous sodium bicarbonate (30 mL) was added, and stirring proceeded at 25 °C for 30 minutes. The mixture was extracted with ethyl acetate (3 \times 50 mL). The combined organic layers were washed with 5 % aqueous sodium carbonate, washed with brine, dried over MgSO₄ and evaporated. The crude product was suspended and stirred in hexanes-ethyl acetate (1:2) for 30 minutes, then filtered. The pale orange solid was collected on a fritted funnel. Yield: 0.807g (60.6 %). ¹H NMR (CDCl₃), δ (ppm) 3.96-4.10 (t, 4H, CH₂N), 4.27 (s, 4H, CH₂CO), 4.37-4.50 (t, 4H, CH₂O), 7.28 (s, 4H, aromatics). ¹³C NMR (CDCl₃), δ (ppm) 39.11 (CH₂CO), 41.10 (CH₂N), 60.39 (CH₂O), 128.44, 130.98 (aromatics), 152.00, 169.77 (C=O).

3.3.9 Synthesis of 2,4,6-triisopropylbenzenesulfonyl azide (Compound V)

Compound V was synthesized according to the procedure described by Dermer and Edmison (16). Sodium azide (0.67g, 99 %, 10.2 mmol) was dissolved in water (5 mL) and acetone (9 mL) was added to the aqueous solution. The mixture was cooled in an ice bath. 2,4,6-Triisopropylbenzenesulfonyl chloride (2.35 g, 97%, 7.5mmol) in acetone (18 mL) was added dropwise to the stirred cold sodium azide solution. The mixture turned pale orange and then pale red in color. The reaction mixture was stirred for 30 minutes in an ice bath and for another 30 minutes at room temperature. By the end of this reaction time the red color had disappeared. The mixture was diluted with water (25 mL), which caused a pale yellow oil to deposit at the bottom of the funnel. The crude product was removed. The water layer was extracted with ether $(3 \times 25 \text{ mL})$. The ether extract and crude oil were combined, washed with water (4×20 mL), dried over sodium sulfate, and evaporated. The colorless oil was crystallized in the freezer. After warming, there was some solvent left in the crude product. The crystals were dried over oil pump and prepared in 2.12 g (90.9 % yield), mp 38-39 °C (lit.: 41-43 °C). IR (Nujol) 2120 cm⁻¹ (N₃) and 1182 cm⁻¹ (SO₂). ¹H NMR (CDCl₃), δ (ppm) 1.21-1.35 [m, 18H, CH(CH₃)₂], 2.93 (m, 1H, para, CH(CH₃)₂], 4.06 (m, 2 H, ortho, CH(CH₃)₂], 7.23 (s, 2H, aromatics).

3.3.10 Synthesis of α,α'-diazido-β,β'-bis(2-oxo-3oxazolidinyl)-β,β'-dioxo-1,4-diethylbenzene (Compound VI)

Compound VI was synthesized according to the procedure described by Falck-Pedersen and Undheim (14). Compound IV (0.914 g, 2.96 mmol) in dry THF (90 mL) was added via a cannula to a solution of 0.5 M potassium bis(trimethylsilyl) amide (13.1 mL, 6.53 mmol) in toluene diluted with dry THF(20 mL) under nitrogen at -78 °C. The resulting solution of the potassium enolate was stirred at -78 °C for 2 hours. Solution of precooled Compound V (2.02 g, 6.53 mmol) in dry THF (15 mL) was added via a cannula. The solution was stirred for 30 minutes at -78 °C before the reaction was quenched by addition of acetic acid (1.6 mL, 0.028 mol). The bright yellow reaction solution was warmed to 30 °C on a water bath and stirred at this temperature for 2 hours. The reaction mixture was diluted with brine (200 mL) and extracted with ethyl acetate (250 mL). The aqueous layer was extracted with ethyl acetate $(2 \times 100 \text{ mL})$. The combined organic extracts were washed with aqueous sodium bicarbonate, dried over sodium sulfate, and evaporated. The bright yellow residue was washed with hexanes. The filtrate was stored in the freezer and then some crystals formed. After filtration, a yellow solid was obtained 0.477 g (46.5 % yield). From the ¹H NMR spectra, the compound contained starting material (Compound VI) and product (1/1). In some syntheses, this mixture was used directly for preparation of the next intermediate. A purified

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sample was prepared by column chromatography in very low overall yield (0.3 %). ¹H NMR (CDCl₃), δ (ppm) 3.96-4.10 (t, CH₂N), 4.27 (s, CH₂CO), 4.37-4.50 (t, CH₂O), 6.22 (s, CHN₃), 7.28 (s, 4H, aromatics), 7.52 (s, aromatics).

3.3.11 Attempted synthesis of α,α'-bis(t-butoxycarbonylamino)-β,β'-bis(2-oxo-3-oxazolidinyl) -β,β'-dioxo-1,4-diethylbenzene (Compound VII)

Two attempts were used to synthesize compound VII according to the procedure described by Falck-Pederesn and Undheim (14). First reaction: Compound VI (0.26g, containing compound IV) dissolved in dioxane

(9 mL) was added dropwise with stirring to a solution of $SnCl_2$ (0.68g) in dioxane (18 mL) and water (9 mL) at 0 °C. The solution was then stirred at room temperature overnight. (Boc)₂O (1.11 g) and NaHCO₃ (0.42 g) were added to the reaction solution. The solution was stirred at room temperature overnight and then acidified with aqueous potassium bisulfate (about pH 3). The reaction mixture was extracted with ethyl acetate (3 × 50 mL). The combined organic layers were washed with aqueous NaHCO₃ (50 mL) and then brine (50 mL), dried over sodium sulfate, and evaporated. After evaporation, a yellow sticky product was left. NMR analysis of the product showed no signal at 5.50 ppm (CHNH.Boc) or 6.13 ppm (compound VI, CHN₃), but there was a signal at 4.27 ppm (compound IV).

The second reaction: Compound VI (0.201g) in dioxane (9 mL) was added

3.3.12 Synthesis of α-amino-phenylacetic acid (Compound VIII)

The synthesis was a modification of the procedures described by Steiger (6). Sodium cyanide (95 %, 52.6 g, 1.0 mol) was dissolved in water (40 mL) and then ammonium chloride (58.8 g, 1.1 mol) was added to the aqueous solution. Benzaldehyde (106g, 1 mol) in absolute methanol (40 mL) was added to the stirred solution. The mixture changed from white to yellow and the temperature rose. The reaction mixture was stirred at room temperature for two hours. The heterogeneous mixture was diluted with water (500 mL) and then extracted with benzene (500 mL). The benzene layer was washed with water $(3 \times 25 \text{ mL})$ and then shaken with concentrated HCl solution (6 M), one 300 mL portion and two 150 mL portions. The combined acidic extracts were heated and stirred under reflux for two hours. The hydrolysate was treated with Norit (about 5 g) and filtered. The yellow filtrate was neutralized with ammonium hydroxide (175 mL) and some yellow crystals formed, as the mixture was cooled to room temperature. These yellow crystals were collected. The crystals were washed with water (500 mL) in small portions, ether (75 mL), hot ethanol (95%, 75 mL) and then water (500 mL). The pale yellow product was dried in a desiccator over CaSO₄.

The crude product was dissolved in NaOH solution (1 N, 400 mL) and then ethanol (95 %, 250 mL) was added. The solution was filtered and the filtrate was heated to the boiling point (about 84 °C). HCl solution (5 N, 80 mL) was added slowly to the boiled filtrate. The mixture formed crystals as it cooled at room temperature. The crystals were collected by filtration and washed with ethanol (95 %, 50 mL) and then water (150 mL). The product was dried in a vacuum desiccator over P₂O₅. The pale, yellow lustrous platelets weighed 45.9 g (30.3 % yield). ¹H NMR (D₂O + DCl), δ (ppm) 4.94 (s, 1H), 7.23-7.25 (s, 5H). The compound is α -aminophenylacetic acid.

3.3.13 Synthesis of α-amino-phenylacetic acid methyl ester. HCl (Compound IX):

 α -Amino-phenylacetic acid (10 g) suspended in absolute methanol (150 mL) was cooled in an ice bath. The reaction mixture was in a three-neck rounded flask, fitted with CaCl₂ drying tube. HCl gas was passed through the mixture. The solid α -aminophenylacetic acid immediately dissolved. After 20 minutes, addition of HCl gas was stopped, the reaction mixture was sealed and stirred at room temperature overnight and then evaporated with a rotary evaporator. As crystals formed in the reaction mixture, the evaporation was stopped. The cloudy mixture was stored in the freezer. After filtration, fine white crystals were obtained: 11.8 g (88.3% yield). ¹H NMR (DMSO-d₆): δ (ppm) 3.71 (s, 3H), 5.27 (s, 1H), 7.44-7.54 (m, 5H), 9.20 (br s, 3H).

3.3.14 Synthesis of 3,6-diphenyl 2,5-diketopiperazine (Compound X)

There are several attempts to synthesize Compound X.

The first attempt: Compound IX (1g, 5 mmol) dissolved in water (1.4 ml) and then triethylamine (0.69 mL, 5 mmol) was added dropwise to the aqueous solution in an ice bath. The aqueous solution changed to heterogenous mixture. Ethanol was added dropwise to the stirred mixture until it changed to homogenous solution. The reaction mixture was stirred at room temperature for 4 days, and then stored in the refrigerator for 4 hours. After chilling, the reaction was filtered. The white crystals were washed with cold water, cold ethanol and then dried. The ¹H NMR spectrum of the crude compound was the same as that of phenyl glycine (Compound VIII). The filtrate was evaporated and some solid was left. The melting point of the solid was the same as that of triethylamine hydrochloride (about 253-254 °C).

The second attempt: Compound IX (1g, 5 mmol) was dissolved in DMSO (3 mL) and then triethylamine (0.69 mL, 5 mmol) was added to the reaction mixture in an ice bath. The reaction mixture became solid. On warming to room temperature, it changed to a clear solution and then to a sticky suspension. The reaction mixture was stirred at room temperature for two days. After two days, the reaction mixture formed some crystals. The mixture was filtered and then washed with ether. The solid was triethylamine hydrochloride (m.p.: about 255 °C). The filtrate was evaporated with an oil pump and a brown solid was left. It is difficult to

remove the solvent (DMSO). The residue was checked with ¹H NMR and no signal (Ph-CH) appeared in the spectrum.

The third attempt: Compound IX (1g, 5 mmol), DMAP (0.1 g) and triethylamine (0.69 mL, 5 mmol) were all suspended in DMF (2 mL). The reaction mixture was refluxed at 90 °C overnight. The solution was cooled in the refrigerator for 4 hours and then filtered. The solid was triethylamine hydrochloride (m.p.: about 255 °C). The filtrate was evaporated with an oil pump. The residue was a brown oil. The crude product was checked with ¹H NMR and no signal (Ph-CH) appeared in the spectrum.

The fourth attempt: Compound IX (1g, 5 mmol) and triethylamine (0.69 mL, 5 mmol) were added to dry ethanol (10 mL). At first, Compound IX was suspended in the solution and then dissolved very well. The reaction mixture was stirred at room temperature for two days and then stored in the refrigerator for 4 hours. After chilling, some crystals formed. The reaction was filtered. The crystals were triethylamine hydrochloride (m.p.: about 253-254 °C). The filtrate was evaporated and some crystals left. The ¹H NMR spectrum of the crude compound was the same as that of phenylglycine (Compound VIII).

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