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Preparation of arylphosphinic acid derivatives as building blocks for binding sites

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Preparation of arylphosphinic acid derivatives as building blocks for binding sites

by

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ABBREVIATIONS

ANS: sodium-1,8-anilinonaphthalene sulfonate

t-Boc: *tert*-butoxycarbonyl

CAC: critical aggregation concentration

Cbz: benzyloxycarbonyl

DABCO: 1,4-diazabicyclo[2.2.2]octane

dist: distilled

EtOAc: ethyl acetate

HBTU: (2-(1-hydroxybenzotriazol-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate

HEPES: N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid

HOAc: acetic acid

MEK: methylethyl ketone

PBP: 4,4'-hydoxyphosphinylidine bis phenylalanine

RT: room temperature

sat'd.: saturated

TFA: trifluoroacetic acid

THF: tetrahydrofuran

TNS: sodium-6-p-toluidinyl-2-naphthalene sulfonate

GENERAL INTRODUCTION

An Explanation of the Dissertation Organization

This dissertation consists of three papers that have been either published or submitted for publication. The papers are preceded by a general background introduction and the research project description and followed by a general conclusion. The references cited in the general introduction follow the general conclusion. Each paper consists of introduction, results and discussion and experimental section. The references cited in each paper follow the experimental section.

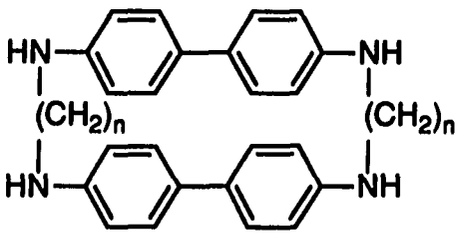
Background

Molecular recognition deals with noncovalent interactions between large molecules (hosts) and substrates (guests). The goal of this research area is to understand the binding of substrates to enzymes and proteins in biological systems and to utilize the binding substances for separation, analysis, structure organization and catalysis of chemical reactions. This is a relatively new field in organic chemistry. In 1938 Freudenberg and Meyer-Delius reported the formation of the adduct between iodine and cyclodextrins inside the cyclodextrin cavity.[1] This was very early evidence for the inclusion of a guest within the intramolecular cavity of a host. At the end of the forties and at the beginning of the fifties, Cramer undertook the first systematic studies on the cavity inclusion complexation between cyclodextrins and organic guests in aqueous solution and in the solid state.[2] The successful studies of natural and modified cyclodextrins by Cramer,[2] Bender,[3] Breslow,[4] Tabushi,[5] and

others[6]have stimulated the later development of fully synthetic hosts and their applications in molecular recognition.

Despite the research on complexation and catalysis of cyclodextrins, the progression to synthetic hosts for organic and inorganic guests did not occur until fifteen years later because of the lack of understanding of the intermolecular complexes formed by enzymes, biological receptors and the natural cyclodextrins. In 1967, the discovery of the synthetic crown ether was remarkable milestone in molecular recognition.[7] Pedersen's study demonstrated that molecular complexation is not limited to natural hosts but also occurs effectively with fully synthetic receptors. Since the discovery of crown ethers by Pedersen, the chemistry of synthetic hosts for the selective complexation of organic and inorganic guests has seen an extraordinarily rapid development.

In 1955, Stetter and Roos first recognized the potential of cyclophanes for inclusion complexation and synthesized the macrocycles **1**,**2** and **3** from benzidine.[8] They reported the formation of inclusion complexes with benzene and dioxane when the macrocycle **2** and **3** recrystallized from those solvents. It was presumed that the guest was included in the cavity of the macrocycles. The presumption was ultimately found to be wrong. Twenty seven years later, this result was corrected by Hilgenfeld and Saenger based on X-ray crystallography. They showed that the large macrocycle **3** and benzene do not form an intramolecular cavity inclusion complex, but a clathrate in which benzene is accommodated between host molecules in the crystal lattice.[9]

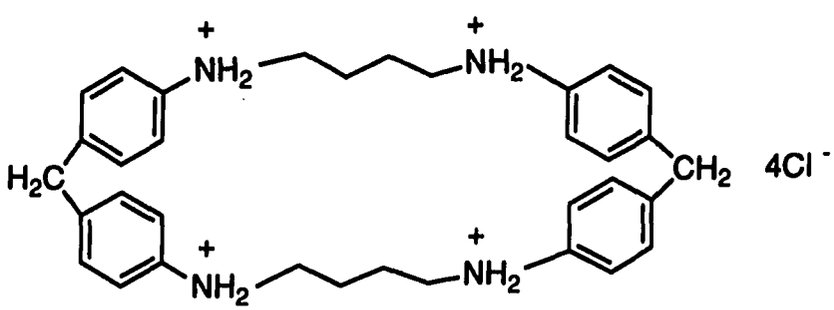


1 n = 2

2 n = 3

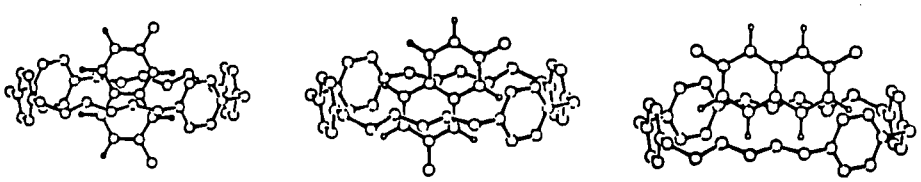
3 n = 4

In the seventies, the research groups of Tabushi,[10] Murakami,[11] Koga[12] and Whitlock[13] started the development of water soluble cyclophane-type macrocycles and analyzed their ability to act as hosts in aqueous solution. Koga et al. replaced the benzidine unit of Stetter's macrocycles by diaminodiphenylmethane units to obtain the 30-membered macrocyclic tetraamine **4**.[12] By NMR spectroscopy, they demonstrated the formation of a 1:1 complex between host **4** and 2,7-naphthalene diol in dilute HCl solution at pH < 2 with an association constant $K_a = 2.8 \times 10^3$ L mol^{-1}. The complex of host **4** and durene, which crystallized from acidic aqueous solution, was characterized by X-ray crystallography as a cavity inclusion complex. For the first time the complete inclusion of an apolar guest in a synthetic host had been proven.



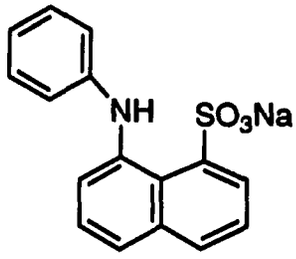
4

^{1}H NMR spectra of the complex of 2,7-naphthalene diol and **4** show strong upfield shifts. From the fact that different protons of the guest are differently shifted, a preferred orientation of the guest in the host cavity has been deduced.[14] A comparison of the upfield shifts observed with those calculated for reasonable geometries leads to the conclusion that the pseudoaxial orientation of the guest is favored to the axial or equatorial orientation. An X-ray analysis of this complex proved the preference for this proposed orientation and confirmed the viability of the NMR method. In contrast to this result, the same guest, complexed with host **5**, two carbon ring members larger in size, is enclosed in an equatorial orientation.[15]

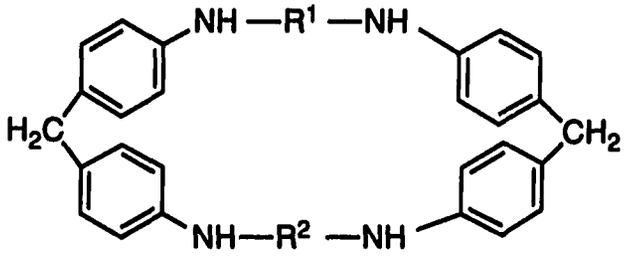


axial pseudoaxial equatorial

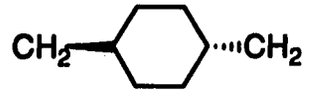
By variation of the bridges between the diphenylmethane units, Koga et al. subsequently prepared a series tetraazaparacyclophanes **5-9** which all dissolve in acidic aqueous solution, and studied their complexation by using 1,8-ANS, a fluorescent indicator, as a probe.[16]



sodium-1,8-anilinonaphthalene sulfonate (1,8-ANS)



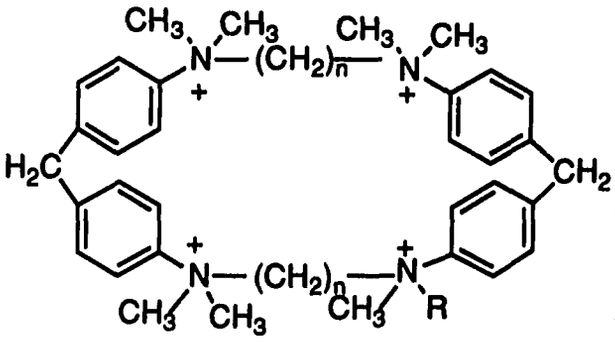
	R^1	R^2
5	$(CH_2)_5$	$(CH_2)_5$
6	$(CH_2)_5$	$(CH_2)_6$
7	$(CH_2)_6$	$(CH_2)_6$

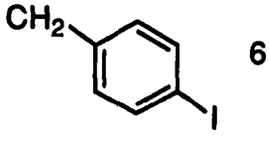


The complex stability increased in the order **4<5<6<<8**. The enlargement of the hydrophobic surface using cyclohexane units is responsible for the fact that **8** exhibits by far the highest complex constant value, an 80 fold increase compared with **4**. Compound **9** showed lower inclusion capability, possibly because of the lower hydrophobicity of a benzene ring than a cyclohexane ring. The diphenylmethane units seem to be essential as they serve as spacers for the cavity and also favor the "face orientation" with the benzene rings turned perpendicular to the macrocyclic ring, which leads to a deeper cavity. The hosts illustrate selectivities towards different guests. The selectivities were studied by qualitative fluorescence measurements.[17] As a result, **4** prefers

binding to β-substituted naphthalenes and **6** and **8** to α-substituted naphthalenes. This can be explained by the orientation of the guests. The guests prefer the pseudoaxial orientation in the small cavity of **4** and the equatorial orientation in the large cavities of **6** and **8**.

Hosts **10**-**12** successfully overcome the disadvantage of **4** which only dissolves in strongly acidic solution. These octamethyl derivatives of **4** are soluble in neutral water solution. ^{1}H NMR titrations and fluorescence spectra give proof of the inclusion capacity of these hosts.

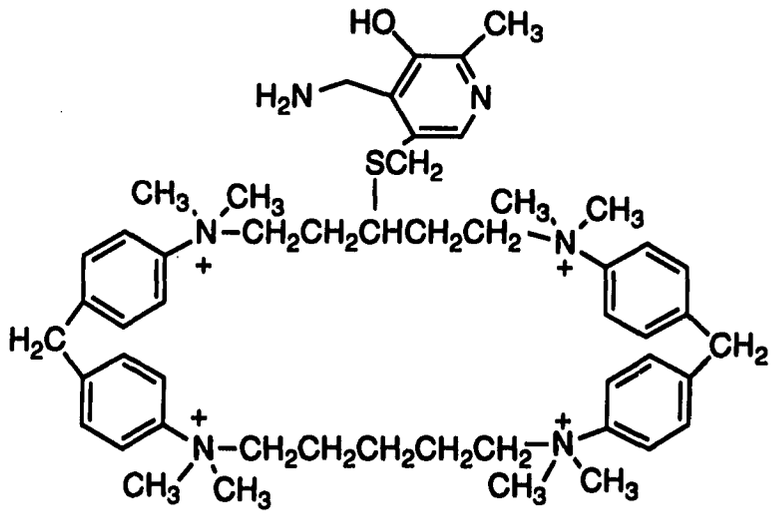


	R	n
10	CH_3	4
11	CH_3	5
12	CH_3	6
13		6

Schneider et al. demonstrated the rate acceleration of nucleophilic substitution catalyzed by host **12** in 1984.[18] Reaction of 2-bromomethyl naphthalene with an excess of sodium nitrite was accelerated in a 0.4 M solution of **12** by a factor of 20; concomitantly, the ratio [R-ONO] : [R-NO_2] obtained with the ambident nucleophilic changed from 0.5:1 to 0.16:1. Both the observed acceleration of the reaction and the product control can be interpreted in terms of an accumulation of nitrite ions at the positively charged centers of **12** in the complex. This led to an increase in the proportion

of S_N2 reactions and thus to increased attack at the nitrogen atom of the ambident anion. Binding of substrates to **12** is needed for catalysis; reaction of benzyl bromide which binds poorly is not accelerated. Competitive inhibition of 2-bromomethyl naphthalene reaction was observed with the more tightly bound 1,8-ANS.

In 1983, Breslow's research group reported the acceleration of transamination by macrocycle **14**, analogous to Koga's macrocycle,[19] and incidentally the first example of a synthetic neutral water soluble hydrophobic binding site. They had previously attached the pyridoxamine unit to cyclodextrin and used it in catalysis of transamination. The macrocycle **14** was made by adding a pyridoxamine unit to the alkyl chain of compound **11**. It has been found that macrocycle **14** converted phenylpyruvic acid **15** to phenylalanine **16** at a rate 31± 3 times as fast as did simple pyridoxamine. In contrast, the conversion of α-ketovaleric acid **17** to norvaline **18** was only 6 ±1 times as fast with **14** as with pyridoxamine. Under the same conditions, the cyclodextrin analogue of **14** accelerated the reaction of **15** by a factor of 15 ± 2 and of **17** by a factor of 2, compared to with the pyridoxamine rates. These data indicate that binding of the phenyl group of **15** into the macrocyclic cavity of **14** contributes significantly to the rate, as it does for analogous cyclodextrin derivatives. Smaller effects were seen for the much less hydrophobic **17**.



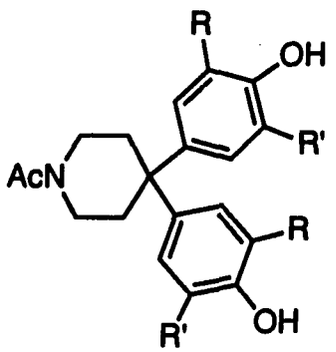
14

15 16

17 18

Diederich and Dick have modified Koga's macrocycles.[20,21] They indicated that hosts described by Koga were not ideal for hydrophobic binding study since the hydrophobic character of these binding sites is perturbed by ionic, strongly hydrated ammonium centers in the periphery of the macrocyclic cavity. For the construction of apolar cavity binding sites shaped by

hydrocarbon residues, they proposed to locate water solubility providing ionic groups remote from the cavity. Compounds **19-21** served as building blocks for these binding sites.

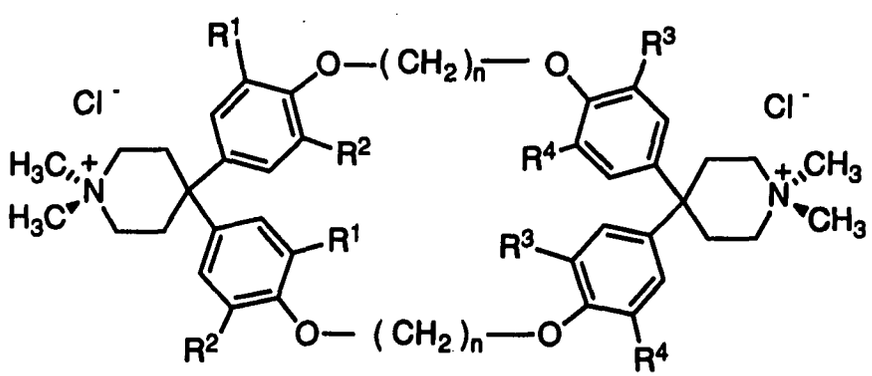


19 R = R' = H

20 R = CH_3, R' = H

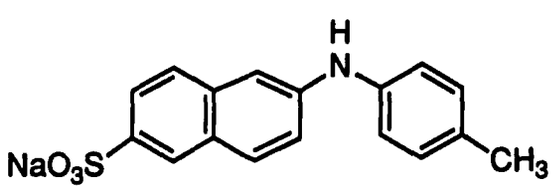
21 R = R' = CH_3

From these building blocks, macrocyclic hosts **22-29** were prepared. In the series **26-29,** two, four, six and eight methyl groups are attached to the hosts. As a result, the cavity becomes successively deeper and more hydrophobic. The hydrophobic cavities of these macrocyclic hosts are no longer disturbed by positive charges and as a consequence enlarged. On the other hand, increased micelle formation and aggregation of the host molecules were observed by shifts of the host protons, without guest being present, in the ^1H NMR spectra depending on the concentration of the host.



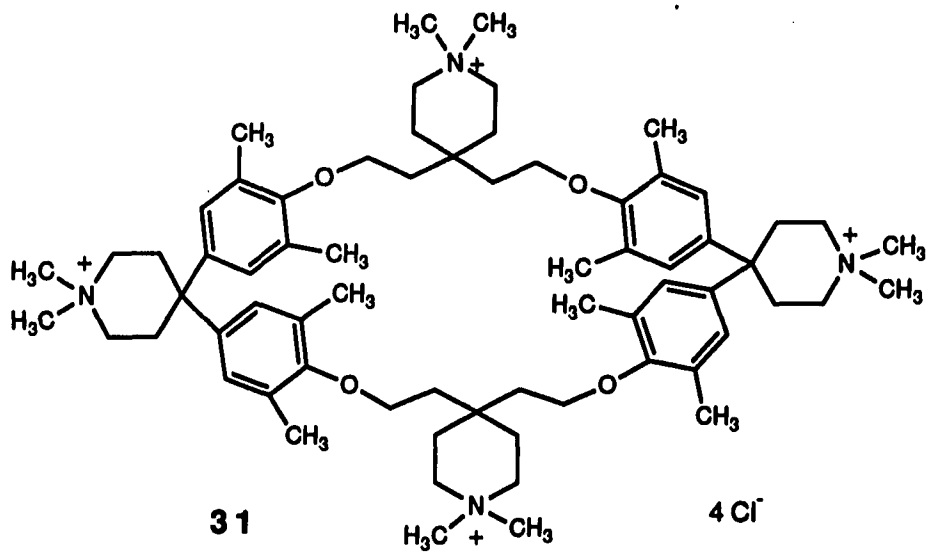
	$R^1=R^2=R^3=R^4$	n		R^1	R^2	R^3	R^4	n
22	H	2	**26**	CH_3	H	H	H	4
23	H	3	**27**	CH_3	CH_3	H	H	4
24	H	4	**28**	CH_3	CH_3	CH_3	H	4
25	H	5	**29**	CH_3	CH_3	CH_3	CH_3	4

The complexation of **24** and TNS **30** was studied by fluorescence spectroscopy at the concentration below the CMC value = $2.5x10^{-4}$ mol L^{-1}. [22] The complex constant is $4.3x10^3$ L mol^{-1}. With 2,7-naphthalene diol as the guest no significant ^1H NMR upfield shifts were observed. Interestingly, removal of the positive charges from the ring does not improve the hydrophobic binding significantly.



sodium-6-p-toluidinyl-2-naphthalene sulfonate (TNS)

These results led to an improved host structure **31**. By attaching spiro piperidinium groups to the bridge chains, compound **31** locates two additional ionic centers away from the cavity binding site. Host **31** has increased molecular-dispersed water solubility. The two additional spiro units also help to enforce the binding site by limiting the number of conformations of the aliphatic bridges.



31 4 Cl^-

The critical aggregation concentration (CAC) of **31** is 7.5×10^{-3} mol L^{-1} which is about 375 times higher than the CMC of host **29** with only two ionic centers.

With the host molecule **31**, Diederich and Dick prepared for the first time a series of 1:1 complexes of aromatic hydrocarbons of variable size in aqueous solution by solid-liquid and liquid-liquid extraction procedures.[21,23] The amount of extremely insoluble hydrocarbon that can be solubilized in water is dramatically increased by host-guest complexation. As an example, the maximum solubility of pyrene in water is only 8×10^{-7} mol L^{-1}. With a 5.5×10^{-3} M aqueous solution of **31** in solid-liquid extractions, however, a solution of 2.8×10^{-3} mol L^{-1} pyrene-**31** complex can be prepared. The association constants of the 1:1 complexes of arenes, determined from the two extraction procedures, are listed in Table 1.

Table 1. Association constants (K_a) and standard free energyies of complexation ($-\Delta G°$) for the 1:1 complexes of the host **31** with aromatic hydrocarbons in water

Guest	K_a [L mol^{-1}]	$-\Delta G°$ [Kcal mol^{-1}]
Perylene	1.6×10^7	9.6
Fluoranthene	1.8×10^6	8.4
Pyrene	1.8×10^6	8.4
Biphenyl	2.2×10^4	5.8
Azulene	2.1×10^4	5.8
naphthalene	1.2×10^4	5.5
Durene	1.9×10^3	4.4

Perylene forms the most stable complex with **31** because perylene has the highest complementarity to the binding site and the largest surface for hydrophobic and Van der Waals interactions. The geometric complementarity between **31** and smaller arenes is less favorable and, expectedly , the complex stability decreases. Perylene binds better than pyrene and fluoranthene and a strong decrease in complex stability was observed with the smaller guests biphenyl, azulene, and naphthalene. Hydrophobic interactions represent the most important driving force for complexation, as shown by the solvent dependency of the binding strength. Strong complexation is only observed in aqueous solution. In methanol, the free binding energy of the **31**-pyrene complex (K_a =75 L mol^{-1}) is 5.9 Kcal mol^{-1} smaller than in water and, in dimethylsulfoxide, almost no complexation at all was observed. Detailed

information about the spatial relationship between the two binding partners in the complexes could be obtained by ^{1}H NMR spectroscopy.[24] In the complexes, the diatropic ring currents of both host and guest mutually influence the positions of their proton resonances and selective complexation shifts were observed. Extensive ^{1}H NMR studies showed that all aromatic guests prefer a location in a specific plane in the cavity of the host **31**.[25] This arene-guest plane of **31** passes through the two spiro carbon atoms of the two diphenylmethane units and perpendicular to the mean molecular plane of the host. An incorporation of an aromatic guest in this plane is easily recognized in the ^{1}H NMR spectrum by a characteristic pattern of upfield and downfield shifts of the host signals as well as by specific upfield shifts of the guest signals.

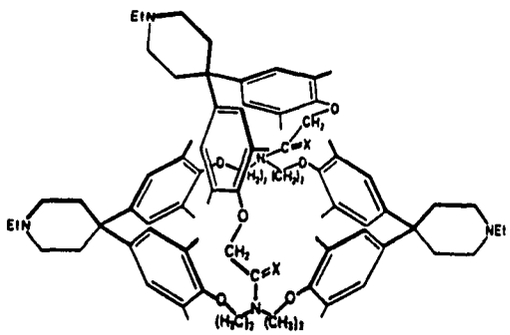
The host **31** is also capable of complexing alicyclic derivatives in aqueous solutions (Table 2).[26]

Table 2. Association constants (K_a) and standard free energies of complexation ($-\Delta G^\circ$) for the 1:1 complexes of the host **31** with alicyclic compounds in water

Guest	K_a L mol^{-1}	$-\Delta G^\circ$ Kcal mol^{-1}
Cyclohexyl acetate	7.6×10^2	3.8
trans-1,4-Cyclohexyanedimethanol	5.0×10^2	3.6
1-Adamantanol	1.6×10^2	2.9

Since the cavity of **31** is quite narrow between the aliphatic chains, only flat alicyclic compounds possess complementary size to the binding size. Hence, **31** forms more stable complexes with cyclohexane than with the more spherical adamantane derivatives.

For a tighter encapsulation of the complexed guests, two macrocyclic hosts **32** and **33** which are soluble in weakly acidic aqueous solutions were prepared. These compounds are designed according to the cryptand concept of Lehn[27] and possess three diphenylmethane units bridged by chains stemming from two "cryptand" nitrogen atoms.[24,28]



32 X=O, **33** X=2H

The comparison of the two hosts **32** and **33** in weakly acidic aqueous solution underscores in an impressive way the importance of locating ionic groups remote from the binding site if strong hydrophobic complexation is to occur. In host **32**, only the three piperidine nitrogen atoms directed outwards are protonated so that the macrocycle is a good host for neutral arenes (Table 3).

Table 3. Association constants (K_a) and standard free energies of complexation ($-\Delta G°$) for the 1:1 complexes of the host **32** with aromatic hydrocarbons in water

Guest	K_a L mol^{-1}	$-\Delta G°$ Kcal mol^{-1}
Pyrene	3.1×10^6	8.7
Naphthalene	1.6×10^4	5.6

In addition to the three piperidine nitrogens, host **33** possesses two tertiary cryptand nitrogen atoms in the periphery of the cavity which are also protonated and favorably solvated so that the hydrophobic character of this binding site is reduced and the complexation of arenes is much weaker. By solid-liquid extraction, the association constant of the **33**-pyrene in 0.5M KH_2PO_4 was estimated as $K_a = 10^4$ L mol^{-1}. The complexation of pyrene in 0.5M KH_2PO_4 by host **33** is therefore about 3.5 Kcal mol^{-1} less favorable than the complexation of this guest by host **32**.

The **32**-pyrene complex is only slightly more stable than the complex of **31**-pyrene, and naphthalene even forms complexes of very similar stability with the two hosts. This high similarity in the complexation properties of two geometrically very different hosts was surprising. The more spherical host **32** was considered to be able to encapsulate guests much better than host **31**. The small difference in complexation ability between **31** and **32** in aqueous solution indicates that the macromonocyclic host **31** already efficiently envelops and encapsulates arenes with complementary size (Figure 1). In addition, favorable solvation of the two amide groups in the periphery of the binding site

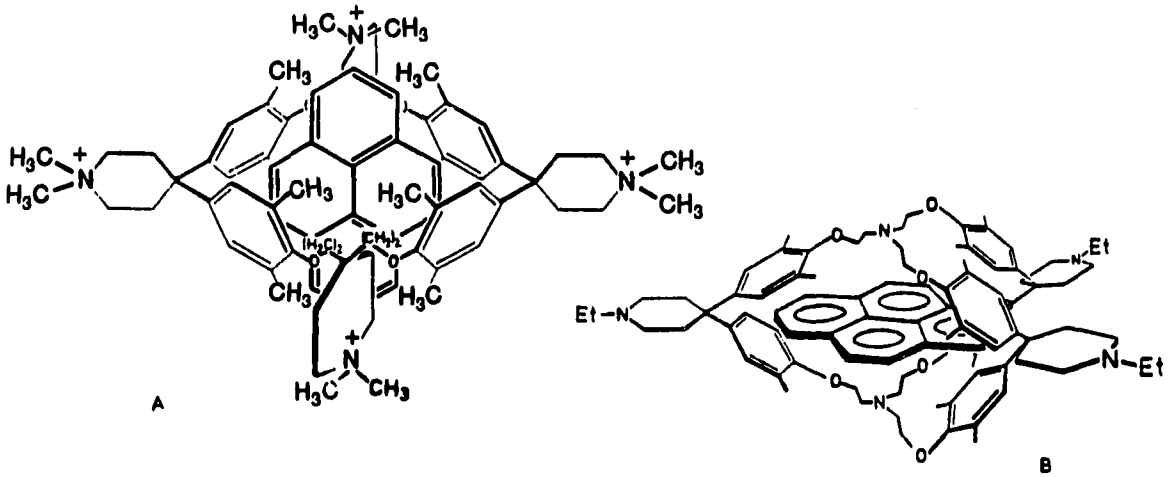
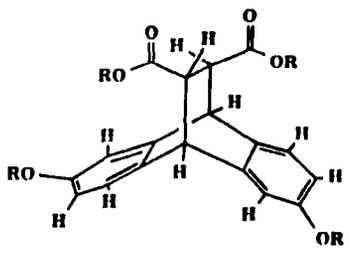


Figure 1. A: complex **31**-pyrene, B: complex **32**-pyrene

of **32** interferes with hydrophobic complexation.

Dougherty's research group also studied hydrophobic binding with the Koga's system. They modified the Koga's system in several important ways to enhance binding and the potential utility of the structures for applications in catalysis and transport. A new class of hydrophobic binding sites was designed and prepared.[29] Compound **34** is the basic building block which arises from a Diels-Alder reaction between dimethyl acetylenedicarboxylate and 2,6-dihydroxyanthracene.[30,31] This building block **34** has an absolutely rigid, concave hydrophobic surface that locks the aryl rings to the "face-to-face" orientation known to be favorable for binding. Connecting two such structures produces an array of four aromatic rings that must be a viable binding site.

This class of host contains many desirable features compared to the Koga's system. The first one is the rigorous separation of the charged groups and the hydrophobic cavity.



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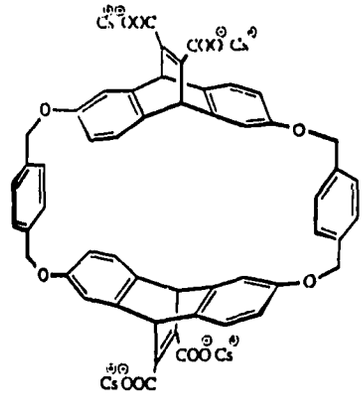


35

This separation increases the binding site's overall hydrophobicity and thus its binding ability. The second is the solubility in neutral aqueous solution. The solubility problem is primarily solved by using quaternary alkylammonium groups or carboxylates. The ester groups of these hosts can be converted to carboxylates which provide water solubility at near-neutral pH. The third feature is the preorganization of the macrocycles. Two 2,6-disubstituted 9,10-ethanoanthracene units have a rigid, concave, hydrophobic surface. The rigid ethanoanthracene also maintains the positions of the carboxylates in a region that is necessarily external to the binding site. Phenols were chosen as the means to introduce the linkers. Also for catalysis development, the ideal host should be able to place functional groups at precise positions around the periphery of the binding site. Since selective functionalization of anthracenes is well known in organic chemistry, the functional group placement should be straightforward in this system. The final issue is chirality. Chirality is a desirable feature that may allow enantiospecific binding and, more importantly, asymmetric induction in

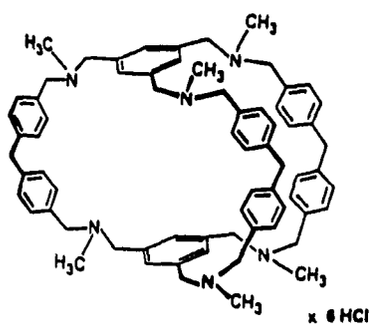
catalysis. This new class of hosts has chirality as a natural feature of the entire structure. An additional advantage of this design is that when the ethanoanthracene building block is obtained enantiomerically pure, macrocyclization produces only one enantiomer of the chiral diastereomers. Thus, after a single resolution, a whole array of chiral hosts varying in the linker positions can be prepared.

Aggregation properties of this class of hosts have been studied by ^1H NMR. All CACs are on the order of 0.2- 0.8mM. Binding studies were carried out in deuterated borate buffer by ^1H NMR. The macrocycles, which were in the form of cesium salts, bound flat aromatic guests with high affinities for anthracene and pyrene Ka = 2×10^6 L mol^{-1}. The hosts have two possible conformations, rhomboid and toroid. ^1H NMR experiments indicate that rhomboid cavity is ideally suited to naphthalene-sized guests in that they fit snugly within the cavity without excess space left. This situation maximizes hydrophobic binding by occluding molecules from the hydrophobic environment of the receptor. The rhomboid conformation is also well suited for π-stacking interactions with flat aromatic guests. The electron-rich aromatic rings of the hosts can lie directly above and below the plane of the bound guest, and the enhanced binding is attributed to strong π-stacking donor-acceptor interactions between the electron-rich host and the electron-deficient quinoline and isoquinoline guests.

Dougherty et al. also made very water soluble N-methylquinolinium and N-methylisoquinolinium as guests.[29] On a per gram basis, these salts are more water soluble than sodium bicarbonate and sodium phosphate. Yet, they are very strongly bound by their hydrophobic receptors. The hydrophobicity of

these guests should significantly reduce the driving force for association with a hydrophobic binding site, and so the similar binding affinities of cationic guests towards hosts relative to their neutral counterparts indicate a substantial enhancement in attractive host-guest interactions. Dougherty and co-workers attributed this enhanced binding to ion-dipole attraction between the positive charge of the guest and the polarizable π-bonds of the host, which can be as significant as 1 Kcal mol^{-1} in binding free energy.

Vogtle and co-workers have prepared a novel and efficient host in a very short synthetic sequence.[32]

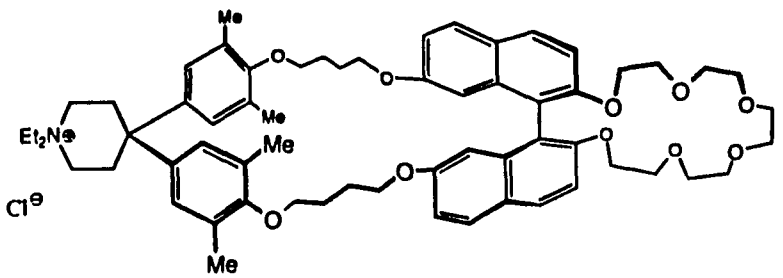


36

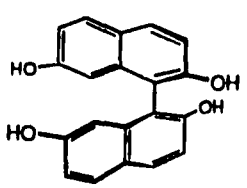
This host **36** which has a symmetrical molecular structure and a large disk-shaped cavity, selectively binds arenes in dilute hydrochloric acid solution. Compound **36** forms stable complexes with polyarenes, such as triphenylene, pyrene perylene and acenaphthalene. Apparently, benzene, naphthalene and azulene are too small and coronene is too large for the cavity. The host **36** shows remarkable binding selectivity to the guests. Phenanthrene is complexed, but the isomeric anthracene is not, a discrimination which has

not been observed even with cyclodextrins. For partially hydrogenated arenes such as hexahydropyrene, dihydrophenathrene, acenaphthalene, and dodecahydrotriphenylene, complexation was not observed with the host **36** even though the corresponding arenes fit in the host cavity. It can be concluded that the cavity of **36** only accommodates such guests in which H atoms of the arene directly project into the rigid angle of the CH_2 group of the diphenylmethane units. This binding selectivity could be exploited for the separation of fluoranthene from a solution of fluoranthene, chrysene, naphthalene, anthracene and adamantane in paraffin oil in 60% yield in single liquid-liquid extraction. Furthermore, it was possible through complex formation with host **36** to separate partially hydrogenated arenes from the parent arenes, e.g. hexahydropyrene from pyrene and dodecahydrotriphenylene from triphenylene.

A cyclophane host **37** with double binding sites has been described by Diederich et al.[33] 2,2,7,7-tetrahydroxy-1,1-binaphthyl unit **38** was used as a potentially efficient, readily available, and versatile chiral spacer for the host **37**.



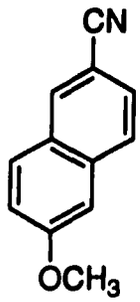
37



38

This ditopic component **38** possesses two distinctive geometric clefts. The minor groove of the binaphthyl shapes an efficient cation binding site with the O-O distance about 3.60 Å. With an O-O distance of about 7.05 Å, the major groove in **37** is almost twice as wide and ideal to shape flat binding sites for arenes.

^{1}H NMR studies showed the efficient inclusion complexation of ten neutral naphthalene derivatives by the host **37** in D_2O/methanol(D_4) mixture in pseudoaxial orientation. The association constant K_a = 4510 L mol^{-1} for the complex **37** and 6-methoxy-2-naphthonitrile **39**.



39

Only a few ditopic hosts are known for which the efficient complexation of two different guests could be unambiguously demonstrated.[34,35] In most cases, the solvent dependency of the two complexation events is very similar. However, a very different solvent dependency could be expected for the complexation of an alkali cation and a neutral naphthalene derivative at the two binding sites of **37**. The association constants of binding of potassium cation and of **39** are shown in the Table 4.

Table 4. Association constants K_a and standard free energies -ΔG for the complexes of host **37** with **39** and K^+

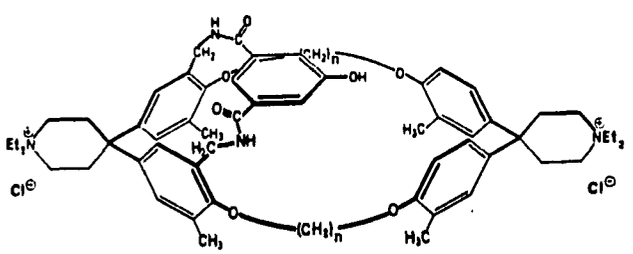
	Guest	D_2O/methanol(D_4) [Vol %]			
		60:40	40:60	20:80	0:100
K_a L mol^{-1}	K^+	13	89	274	1770
-ΔG° Kcal mol^{-1}	K^+	1.5	2.61	3.27	4.35
K_a L mol^{-1}	**39**	4510	441	142	24
-ΔG° Kcal mol^{-1}	**39**	4.90	3.52	2.88	1.85

Clearly, the host **37** constitutes a ditopic receptor which can be switched from an efficient binder of neutral naphthalene derivatives to an efficient binder of potassium cations by simply changing the water content of the water-methanol mixture. Although specific shifts in the ^1H NMR spectra indicate a considerable change in the conformation of the binaphthyl unit upon complexation of a potassium cation, the complexation ability of the major groove binding site is only weakly affected by the cation binding at the minor groove.

One of the ultimate goals of molecular recognition is the development of bioorganic catalysts. Cyclophanes with binding sites for neutral molecules have been designed and studied in an increasing number as enzyme mimics.

Hydrolase mimics have always been at the center of this bioorganic approach toward catalysis. At the active sites of proteases, highly efficient catalytic mechanisms are generated through a specific array of functional

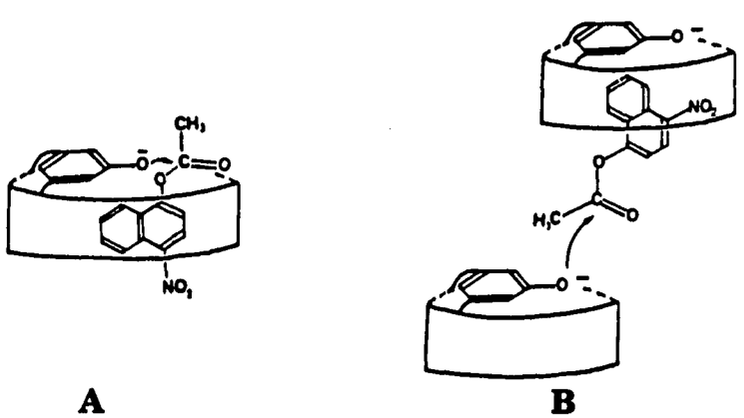
groups. The isolated forms of these functionalities demonstrate little catalytic activity.[36,37] Only by the cooperative action of these groups in the specific active sites catalytic mechanisms such as transition-state binding and stabilization, acid-base catalysis, nucleophilic and electrophilic catalysis and desolvation become effective. Diedrich's research lab has defined α -chymotrypsin mimics as their target systems.[38] These mimics should have features (a) complex their substrates prior to reaction, (b) react according to the minimum mechanism for the α-chymotrypsin- catalyzed hydrolysis of esters and amides, and (c) operate like the enzyme by the addition-elimination mechanism in both transacylation and deacylation steps. They have prepared the macrocycles **40** and **41** which possess phenolic hydroxyl groups as nucleophiles that are active at near physiological pH.



40 n=4, **41** n=6

The nucleophiles are positioned in a well-defined way atop the macrocyclic binding sites. This favorite location of phenolic ring is supported by CPK molecular model examinations and ^{1}H NMR spectroscopy. Both macrocycles form complexes of similar stability with naphthalene derivatives in aqueous solutions. With 4-nitro-1-naphthylacetate as a substrate, a large

difference in the transacylation rates with **40** and **41** was observed. In aqueous phosphate buffer at pH 8.0 (T=20°C, [host] = 5.0×10^{-4} mol L^{-1}, [substrate] = 2.0×10^{-5} mol L^{-1}) the acylation of **40** by the naphthyl ester is only 14 times ($k_{obs} = 2.43 \times 10^{-4}$ s^{-1}) faster than the hydrolysis in the pure buffer. Under the same conditions, the acylation of **41** by the same naphthyl ester is 178 times faster ($k_{obs} = 3.21 \times 10^{-3}$ s^{-1}) than the hydrolysis in the pure buffer. For the acyltransfer in the presence of **41**, saturation kinetics was observed, whereas the reaction in the presence of **40** strictly follows second order kinetics. Only host **41** forms a productive complex with a favorable proximity between the phenolic hydroxyl group and the substrate carbonyl group. The smaller host **40** also forms a stable complex with the naphthyl ester. In this complex, however, the phenol ring completely blocks one side of the cavity. The ester residue of the guest extends in a non-productive manner out of the cavity on the side opposite to the nucleophile and undergoes an intermolecular reaction with the phenol group of another host molecule (Figure 2).



A **B**

Figure 2. **A**: productive binding, **B**: nonproductive binding

The phenolic OH group in **41** is not only a good nucleophile in the transacylation step but, with its estimated pKa ~8.4, also represents a favorable leaving group in the deacylation step as indicated by a weak but significant catalytic turnover. With [**41**] = 1.0×10^{-5} mol L^{-1} and [4-nitro-1-naphthylacetate] = 1.0×10^{-4} mol L^{-1} under conditions mentioned above, the covalently catalyzed ester hydrolysis is accelerated by a factor of 1.3 compared to the hydrolysis in the pure buffer. Under these conditions, only 15% of the total amount of host is complexed.

A major advantage for the development of bioorganic catalysts is the easy and selective functionalization of existing hosts. Diederich and co-workers modified their cyclophanes, especially at the aromatic rings, to obtain the thiazolium derivative **42** in order to explore the influence of an apolar macrocyclic cavity binding site on the activity of thiazolium ions in the benzoin condensation (Figure 3).[39]

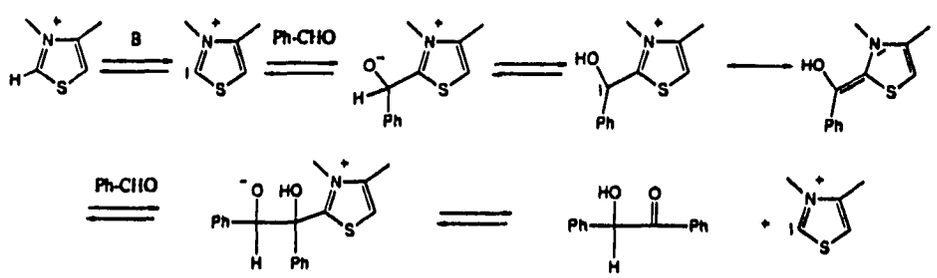
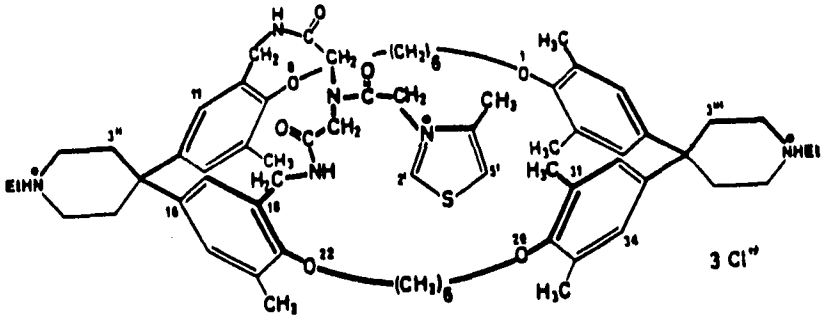


Figure 3. The formation of benzoin from benaldehyde condensation



42

In this macrobicyclic system a thiazolium ring is attached in a favorable position to the binding site which provides space for the two benzaldehyde molecules to react to give benzoin. The entropically favorable proximity and orientation of the substrate and the coenzyme model were expected to affect the catalysis.

The macrobicyclic compound **42** represents a very complete model system for thiamine pyrophosphate-dependent ligases. In protic solvents, where complexation of benzaldehyde takes place, **42** is a better turnover catalyst (k_{cat} =1.1 min^{-1} in methanol) for the benzoin condensation than non-macrocyclic thiazolium derivatives. The observed saturation kinetics could be best evaluated by assuming the formation of a 1:2 complex. In methanol, the equilibrium of the benzoin condensation is far on the side of benzoin, and in reactions catalyzed by **42**, benzoin could be isolated in 93% yield. In DMSO, the equilibrium is far less on the side of benzoin, and by starting with pure benzoin, benzaldehyde could be isolated. A strong influence of the cavity of **42** on the H/D exchange has been observed. The H/D exchange is considerably faster at C-2 of the thiazolium ring of **42** than at C-2 of thiazolium derivatives

without a macrocyclic binding site. This H/D exchange rate enhancement is explained by a micropolarity effect of the cavity of **42** on the kinetic acidity of the proton 2-H of the thiazolium ring. Therefore, one of the catalytic advantages of **42** relative to non-macrocyclic thiazolium derivatives could be the formation of a larger amount of reactive ylide at a given pH.

The above brief review discusses in particular the contributions provided by synthetic cyclophanes as hosts to the understanding of molecular complexation of organic guests in aqueous solution. Many other types of synthetic receptors have been prepared and demonstrated fascinating binding properties. The catalytic studies described above were inspired by very similar work of Breslow with cyclodextrin derivatives. One of the aspects of research in the field of molecular complexation and catalysis is the multidisciplinary approach to generate interesting research questions and to subsequently target them in experiments. Inspiration is provided by studying problems of interest and importance in biological sciences. Open fundamental questions are recognized which can be better answered with the methods of molecular based chemical science. In addition the intensive understanding the extraordinary properties of biological systems such as enzymes and receptors stimulates a strong desire to generate similar properties in designed systems. In this relatively young area of molecular recognition, many interesting questions have been opened in the last twenty five years which need to be answered for a better understanding of the principles of molecular recognition and catalysis.

It can be seen from the structures of the synthetic hosts described as yet that they all contain similar structural building blocks like the

diphenylmethane unit and aliphatic $(CH_2)_n$ bridges. But in principle, there is no restriction regarding these building blocks as long as they are spacers preforming a cavity and units that allow water solubility.

Much of the selectivity seen in binding to the synthetic hosts is dependent on cavity size, even though cavity shape, charge and degree of saturation of substrate are also important. Compared to biological systems, the binding selectivity of synthetic receptors is low. Subtle structural modifications of these receptors to change their binding selectivity are difficult to make.

Multiple interactions in the complexes of these hosts allow for high guest selectivity. It is unknown how the complexing ability of one binding site is influenced by a second, adjacent binding site and its solvation pattern. An improved understanding of multiple interactions in molecular complexes will benefit the rational development of optically active hosts which discriminate effectively between racemic guests.

Research in the field of bioorganic catalysis will lead to compounds with specific advantages such as superior stability and broad applicability. These synthetic catalysts could recognize a large variety of substrates and accelerate many reactions for which no biological catalysts are available. The most worthwhile objective of the current research efforts on the way to such catalysts is to learn how to generate the efficient principles and mechanisms of enzymatic catalysis in synthetic organic systems.

Molecular Design

The long term research project carried out in our lab is to prepare a new class of synthetic binding sites which offers a flexible approach to the problem

of specific molecular recognition. The binding sites are designed for complexing aromatic substrates with hydrophobic interaction.

In the entire field of molecular recognition, two principles have been found to be of special importance for the successful design of strongly binding host molecules: the principle of stereoelectronic complementarity between host and guest and the principle of preorganization of a binding site prior to complexation.[40] The first one essentially represents a modern formulation of the lock-and-key principle.[41] Since we chose aromatic hydrocarbons and derivatives as substrates, the hosts we construct must possess a hydrophobic cavity. In the previous studies of cyclophanes, the diarylmethane unit has been used as rigid aromatic walls for inclusion of hydrophobic guests. Instead of using diarylmethane units, methyl diarylphosphinates would be useful building blocks for our binding sites. By removal of the methyl ester group, the charged oxygens on the exterior of the building block provide water solubility, without interfering with the low polarity of the interior of the molecule (Scheme 1).

Scheme 1.

Simple diarylphosphinates such as methyl bis-*p*-hydroxyphenylphosphinate (in Scheme 1, R=OH) are closely analogous to the precursors of many known macrocyclic binding sites.[40,42] A significant feature

of these diarylphosphinates which we were going to make is the ease with which derivatives may be assembled, bearing variously sized and shape aromatic walls to vary the binding site. Easily increasing the size of such walls, for example from benzene to naphthalene rings [43] will allow binding to much larger guests, such as steroids.

The second principle states that the preorganization of a host prior to complexation is an essential factor controlling the association strength. If a host binding site is not completely organized, a reorganization must occur upon complexation of the guest. This reorganization can cost part of the free binding energy. In the most unfavorable case, the energy needed to reorganize the binding site is larger than the free energy of complexation and no binding will occur. Cram et al. showed that the guest selectivity increases with increasing preorganization of a binding site.[44] In a recent review, Schneider indicated that it is important to take into account the fact that spherical particles are subject, in a hemispherical cavity, to four times as much dispersive binding force as on a planar surface, and that this factor increases to six with a cylindrical cavity and approximately eight with a fully encompassing spherical cavity.[45] In the previous study of cyclophanes, functionalized methylene groups are commonly used as linkers to assemble diarylmethane units to a macrocycle. One of the disadvantages of this type of linker is the difficulty of adding or modifying functional or catalytic groups on the linker. We proposed to connect our building blocks into a cyclic structure by peptide units or through metallic complexation. The peptide linkers have the following features. (a) Certain kind of aromatic amino acid can be readily converted to diarylphosphinates. (b) Peptide bond formation is well known in

peptide chemistry. Many well developed coupling procedures can be used. (c) Peptide linkers are less hydrophobic than methylene chains so that the CAC of the resulting macrocycles could be increased. (d) Starting with an optically pure amino acid, through a series of careful steps, an optically pure macrocycle may be obtained. (e) It is easy to add or change functional groups on the peptide linker. (f) Changing the peptide chain length results in different size of cavity. (g) Incorporate the building blocks into a known peptide sequence may generate a potential DNA binding unit.

The synthesis of the macrocycles we designed allows us to obtain many binding site candidates.

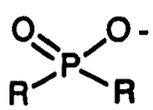
Objective

Since I was one of the people who first started this molecular recognition project, I chose the synthesis of building blocks as my research starting point. The target sof my research were to develop a general synthetic method for preparing arylphosphinates, methods for assembly these building units into designed binding sites and a screening assay for evaluating binding site candidates. Following is the discussion in detail about how I approached these targets.

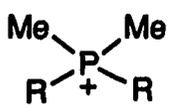
PAPER I. PREPARATION OF ARYLPHOSPHINATES AS BUILDING BLOCKS

INTRODUCTION

In the design of our water soluble binding sites which contain a hydrophobic cavity, diaryl phosphinates were chosen as building blocks for the binding site construction. There are important reasons why phosphinates are being used. First, the anionic phosphinate provides water solubility to binding sites, enabling the study of hydrophobic interaction. Second, compounds which either possesses a negative charge adjacent to the phosphorus atom, as in the phosphinate anion, or a positive charge positioned on the phosphorus atom, as in the quaternary phosphonium salt, can be obtained from phosphinate esters by hydrolysis or reduction and alkylation respectively. Thus we will be able to study both anionic and cationic binding sites.



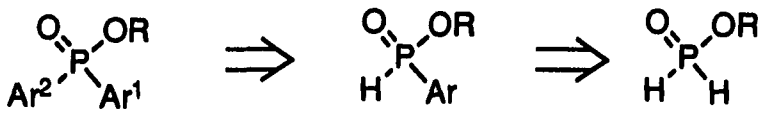
anion



cation

The third reason is that once we have a method to synthesize the phosphinate building blocks, we should be able to build up molecules containing a hydrophobic cavity easily with various sizes and functional groups. The construction of carbon-phosphorus bonds has been studied for decades, but we have not seen any report about the synthesis of monoarylphosphinate **2** with various functional groups. Many procedures for making diarylphosphinates **1** from monoarylphosphinates **2** have appeared in the literature. Since the monoarylphosphinate **2** precursors are not available these procedures could

not be used in our synthesis until we had developed an improved preparation of **2**. The development of a general method for the preparation of monoarylphosphinates will not only benefit our research but also enhance the utilization of many known procedure for making diarylphosphinates.



1 **2** **3**

R = alkyl, H

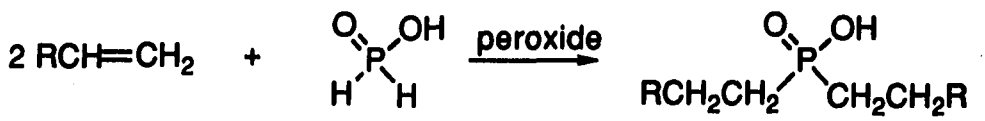
Scheme 1.

Arylphosphinic acid esters **2** have been shown to be precursors to phosphonate monoesters,[1] to phosphonate diesters difficult to make by other routes,[2] and to alkyl, alkenyl and aryl phosphinates which are useful as herbicide intermediates.[3]

Following is a brief review of the known procedures for the synthesis of monoaryl and diarylphosphinates or phosphonates.[4]

Goncalves reported the preparation of phenylphosphinic ethyl esters by esterification of the phenylphosphinic acid with triethyl phosphite.[5] The same author also made phenylphosphinic methyl ester by methanolysis of phenyl dichlorophosphine with methanol.[5] Hewitt introduced a method of making phenylphosphinic ethyl ester by treatment of phenylphosphinic acid with ethyl chloroformate in the presence of a tertiary amine.[6] An aryl group bound to phosphorus is usually derived from a Grignard reagent or similar

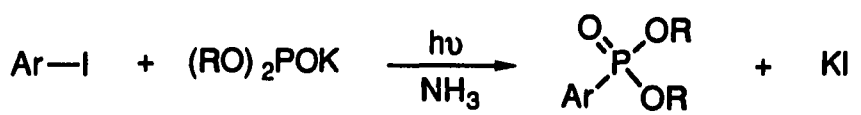
organometallic, or by $AlCl_3$ catalyzed reaction at high temperature, such as making diphenylphosphinous chloride from chlorobenzene and elemental white phosphorus at 350°C.[7] In addition, alkylphosphinic acids may be derived from phosphinic acid by radical addition to alkenes. One of the examples of this type of reaction is the formation of dialkylphosphinic acid by addition of phosphinic acid to olefins in the presence of peroxide.[8]



Scheme 2.

All of these routes are particularly limited by the functional groups compatible with the transformations. Palladium catalyzed reactions for connecting aryl groups and phosphorus atoms are carried out under mild conditions.

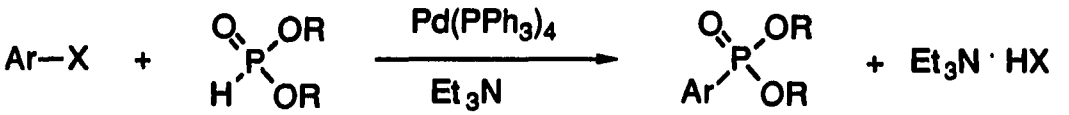
The preparation of arylphosphonic acid diesters has been reported by an $S_{RN}1$ process (Scheme 3).[9] Aryl iodides react rapidly with potassium dialkyl phosphites in liquid ammonia under UV irradiation to form dialkyl arylphosphonates in good yields.



Scheme 3.

The same type of compounds were also made from a palladium catalyzed route (Scheme 4). Hirao reported that dialkyl phosphonates were prepared

from the reaction of arylbromide or iodide with dialkyl phosphonate in the presence of triethylamine and a catalytic amount of tetrakis(triphenylphosphine)palladium.[10] In this route, diethyl arylphosphonates bearing a variety of electron-withdrawing and electron-donating substituents were prepared in high yields.

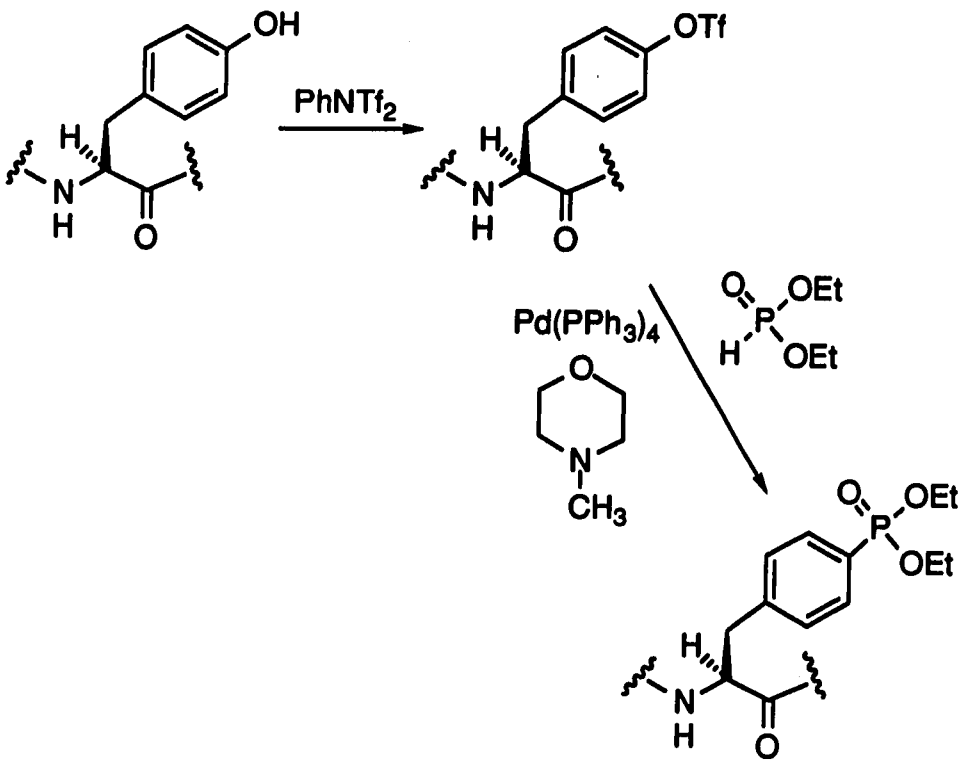


X = Br, I

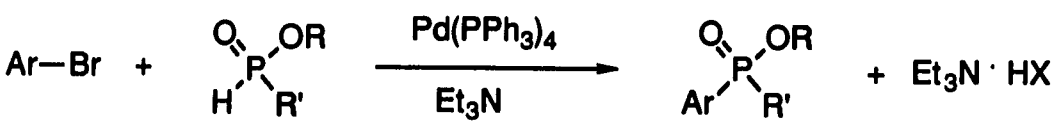
Scheme 4.

Petrakis and coworkers have shown this palladium catalyzed route to be compatible with functional groups present in peptides and also extended this application from aryl bromides or iodides to aryl triflates.[11] In their procedure (Scheme 5), the tyrosine hydroxyl group was converted to triflate and subsequently replaced by a diethoxyphosphinyl group in this tyrosine-containing peptide without detectable racemization. As the author claimed, this process is the first formation of an aryl C-P bond in a peptide framework.

Xu and coworkers demonstrated the synthesis of alkyl alkylarylphosphinates from the reactions of aryl bromides with monoalkyl alkylphosphonites in the presence of triethylamine and tetrakis(triphenylphosphine)palladium (Scheme 6).[12] This procedure can tolerate a wide variety of functional groups such as nitro, ketone, ester and amine groups.



Scheme 5.

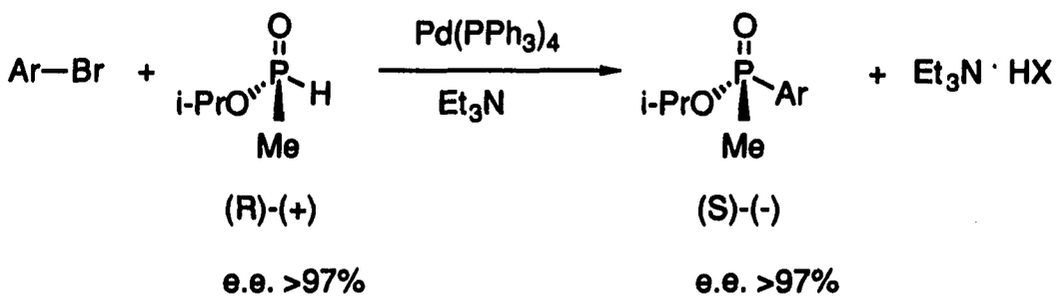


R = CH_3, n-C_4H_9

R'= n-C_4H_9, C_2H_5

Scheme 6.

Xu's research group also showed the palladium catalyzed process proceded with complete retention of configuration (Scheme 7).[13] From their finding that (S)-(-)-isopropyl methylphenylphosphinate was obtained from (R)-(+)-monoisopropyl methylphosphonite in 97% e.e., they suggested that this reaction might be developed into an efficient method for synthesis of optically active phosphinates.



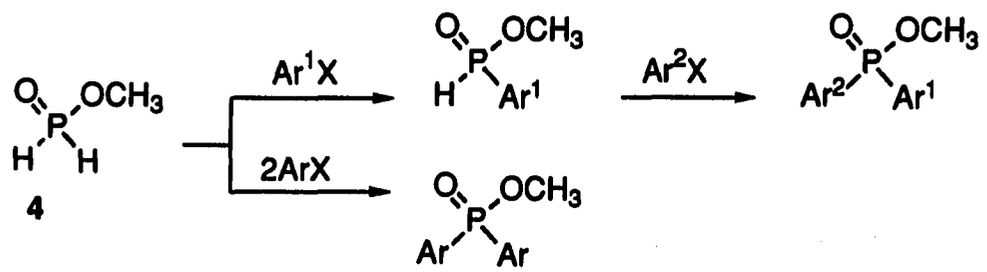
Scheme 7.

A variety of similar palladium catalyzed reactions are known, wherein a halide or triflate is displaced by a nucleophile.[14-17] These reactions have demonstrated that mild palladium catalyzed route is compatible with aryl groups bearing many functionalities. This is the reason we chose a palladium catalyzed route for our synthesis.

In the construction of our designed binding sites, we needed phosphinates bearing either two identical aryl groups or two different aryl groups. These phosphinates with useful functionalities are derived from the corresponding monoarylphosphinates via a palladium catalyzed pathway. Unfortunately, the procedures for making these monoarylphosphinates are not available. Development of a convenient, mild and general method for the synthesis of the

methyl monoarylphosphinates for the preparation of symmetrical and unsymmetrical methyl diarylphosphinates became an essential part of our research.

The ideal synthetic route would be a two step reaction. The first step can be easily controlled to obtain methyl monoarylphosphinates from methyl phosphinate or symmetrical methyl diarylphosphinates. The second step is a conversion of monoarylphosphinates to unsymmetrical diarylphosphinates.



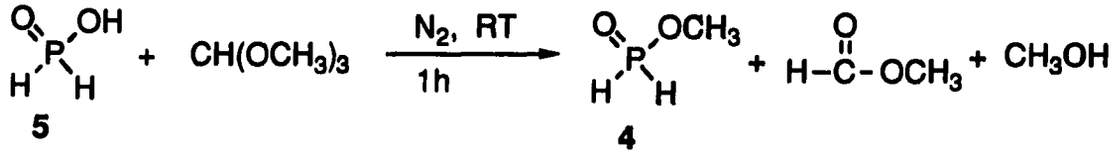
Scheme 8.

In principle, a palladium catalyzed coupling of methyl phosphinate to aryl iodides could provide the desired methyl arylphosphinates. However, there were some concerns about this method. The major problem is that methyl phosphinate **4**, which is not stable even at room temperature, could decompose at the temperatures used for palladium catalyzed reactions. The other problem is that phosphinic acid, a more powerful reducing agent than phosphonic acid, is known to reduce active aryl halides to arenes.[18] Phosphinate salts, in the presence of transition metal catalysts, are known to reduce ketones, nitriles, and nitro groups.

RESULTS AND DISCUSSION

Mark Stoakes, a undergraduate student in our lab, started this project first. He found that iodobenzene and methyl phosphinate in the presnce of $(Ph_3P)_4Pd$ led to diphenylphosphinate in low yield in different solvents and at various temperatures. With this hint that the route was feasible, I stepped in. We dicided to optimize the procedure.

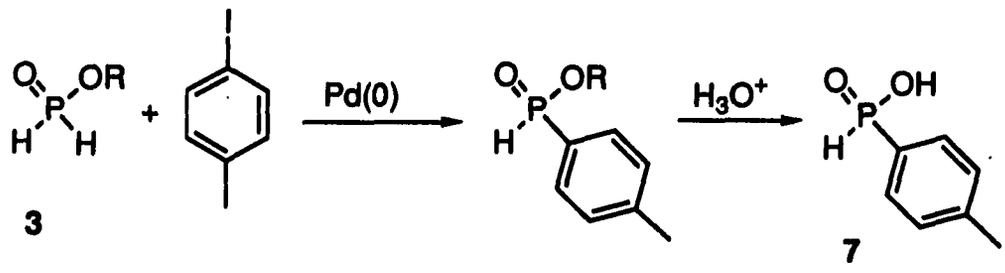
The synthesis of methyl arylphosphinates started by making methyl phosphinate **4**. Methyl phosphinate **4** was prepared by treatment of anhydrous phosphinic acid **5** (hypophosphorous acid) with trimethyl orthoformate.[19]



Scheme 9.

It has been reported that methyl phosphinate **4** thermally decomposes even at 0°C and it is hydrolyzed easily in the presence traces of water[19] so the reaction of phosphinic acid with trimethyl orthoformate was carried out under N_2 atmosphere at room temperature. The product methyl phosphinate was carried on to the next reaction immediately. Our ^1H NMR experimental results showed that methyl phosphinate **4** in trimethyl orthoformate solution is more stable than in its neat form.[20] In methyl orthoformate solution **4** can be kept for days.

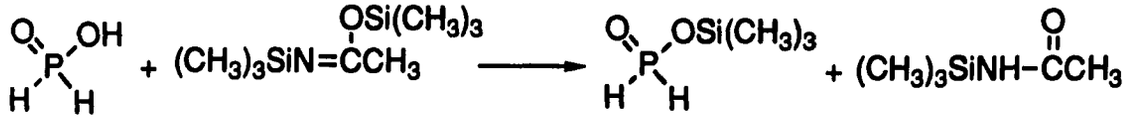
We first tried to improve the reaction by studying other esters of phosphinic acid in a model reaction with iodotoluene.



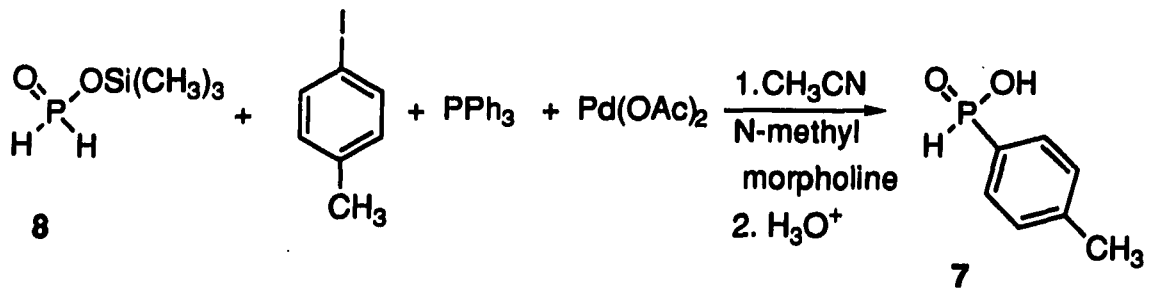
3 7

The trimethylsilyl ester of phosphinic acid **8** was obtained from the treatment of phosphinic acid with 1 equivalent of bis(trimethylsilyl)acetamide in acetonitrile at room temperature. Trimethylsilyl phosphinate reacted with iodotoluene (0.66 eq), triphenylphosphine (0.13 eq), palladium acetate (0.033 eq) and N-methylmorpholine (0.88 eq) in 10 ml CH_3CN. After the coupling reaction trimethylsilyl ester would be cleaved in acidic solution to give arylphosphinic acid, the desired product. This reaction gave an trimethylsilyl arylphosphinate. Unfortunately, the yield of tolylphosphinic acid **7** was about 3%.

Phosphinic acid tributyltin ester **9** was made by the reaction of phosphinic acid with 0.5 equivalent of bis(tributyltin)oxide in toluene at room temperature. Tributyltin can also be cleaved easily by hydrolysis.

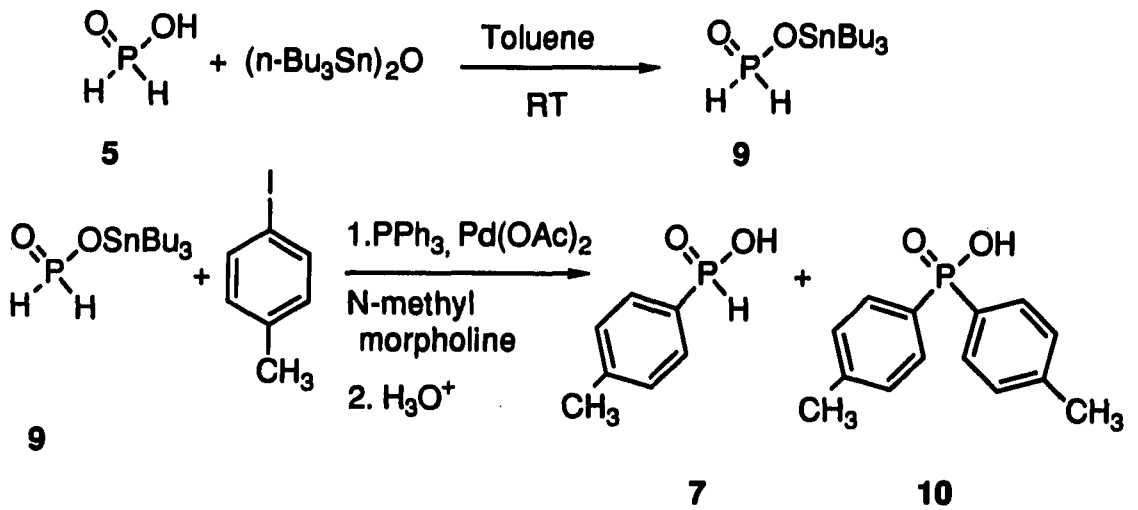


5 8



Scheme 10.

The tributyltin ester **9** was carried on to couple with iodotoluene (0.67 eq) in the presence of triphenyl phosphine (0.13 eq), palladium acetate (0.033 eq), and N-methylmorpholine (0.68 eq) in hot acetonitrile. After the hydrolysis, both monotolylphosphinic acid **7** and ditolylphosphinic acid **10** were formed, but in poor yields (total yield 24%).

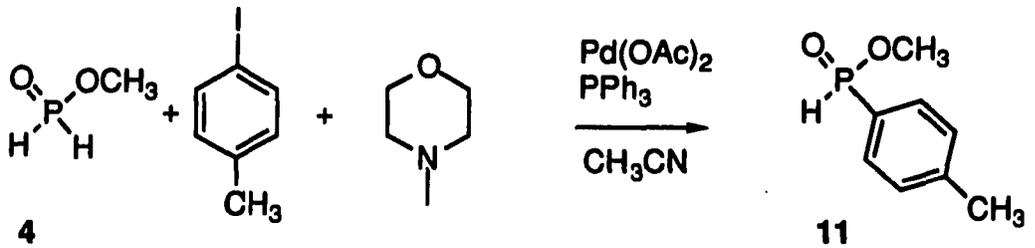


Scheme11.

When we tried trimethylsilyl and tributyltin esters, the optimal reaction conditions had not been found. The monoarylphosphinates were not obtained from the reaction of aryl iodide and phosphinic methyl ester. So we could not

compare the difference between three esters. We decided to use the reaction of methyl phosphinate with iodotoluene as a model system to optimize the reaction conditions for the perparation of monoarylphosphinates since other esters did not provide improved results. The reason of using the synthesis of methyl tolylphosphinate as a model reaction is that its ^1H NMR spectrum can be easily distinguished from that of iodotoluene and other impurities. As results of many experiments, a convenient standard set of conditions for methyl mono- and diarylphosphinate reactions has been chosen. Following is the discussion of these conditions in detail.

(1) The hydrolytic sensitivity of methyl phosphinate requires scrupulously anhydrous conditions and exclusion of air. The reason why this is important is that phosphinic acid, the hydrolyzed product of methyl phosphinate, appears to modify the reaction such that aryl iodide is consumed without forming P-C bonds. Deiodinated protected phenylalanine has been isolated from a reaction of Boc-phenylalanyl methyl ester that gave a low yield of the diarylphosphinate product. Palladium catalyzed reaction of *o*-dibromobenzene with diethylphosphonate has been similarly reported to give diethyl phenylphosphonate.[10] Addition of one equivalent of H_2O based on iodide to the reaction mixture decreased the yield of methyl tolylphosphinate to 13% even though a twofold excess of methyl phosphinate remained in the reaction. Water scavengers such as bis(trimethylsilyl)acetamide (BSA) and molecular sieves have been used, but they did not always improve the yields of these arylphosphinate reactions. It has been found that the best way to keep the reaction from H_2O is to dry glassware and solvent carefully and run the reaction under N_2 (Table 1).



4 11

Table 1. The effect of water scavengers in the methyl tolylphosphinate reaction

Amount of 4	T °C	Reaction time	H_2O scavenger	Yield of 11 (NMR integration)
5 eq	80	1 h	BSA	65 %
4 eq	80	1.5 h	Molecular sieves	19 %
6.6 eq	80	50 min	None	80 %

(2) Anhydrous phosphinic acid is relatively stable when stored in a refrigerator for several months. It may decompose in long time storage. Even though methyl phosphinate is more stable in trimethyl orthoformate solution than in neat form, it still decomposes at room temperature. Methyl phosphinate was always carried on to the next reaction within one hour.

(3) Several ways of carrying the reaction of methyl phosphinate and aryl iodide have been used (Table 2).

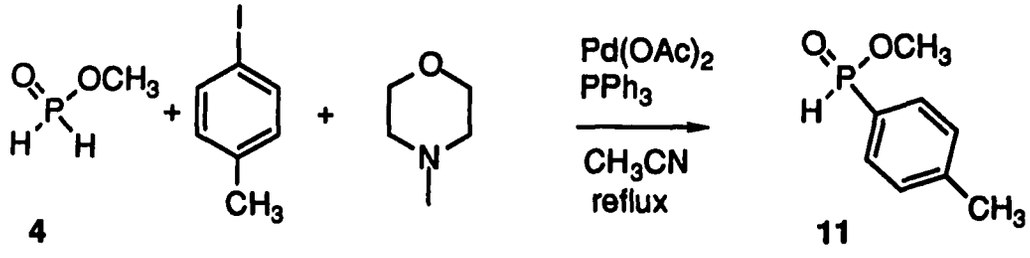
(a) Co-products, methyl formate and methanol, were separated from methyl phosphinate. Then methyl phosphinate redissolved into trimethyl orthoformate and CH_3CN solution. This solution was slowly added over one hour into a refluxing CH_3CN solution containing aryl iodide, palladium catalyst and tertiary amine.

(b) A mixture of methyl phosphinate and co-products was slowly added over one hour into a refluxing CH_3CN solution containing aryl iodide, palladium catalyst and tertiary amine.

(c) CH_3CN solution containing aryl iodide, palladium catalyst and tertiary amine was added in 15 min into a solution of methyl phosphinate (no co-products), methyl orthoformate and CH_3CN. Then the solution was refluxed.

(d) Aryl iodide was added into trimethyl orthoformate and CH_3CN solution containing methyl phosphinate (no co-products). This resulting mixture was added in 40 min into a refluxing CH_3CN solution containing palladium catalyst and tertiary amine. Then the mixture was refluxed.

(e) The reaction mixture of methyl phosphinate and co-products was quickly added to CH_3CN solution containing aryl iodide, palladium catalyst and tertiary amine at room temperature. Then the solution was refluxed.



4 11

Table 2. Comparison of 5 methods in methyl tolylphosphinate reaction

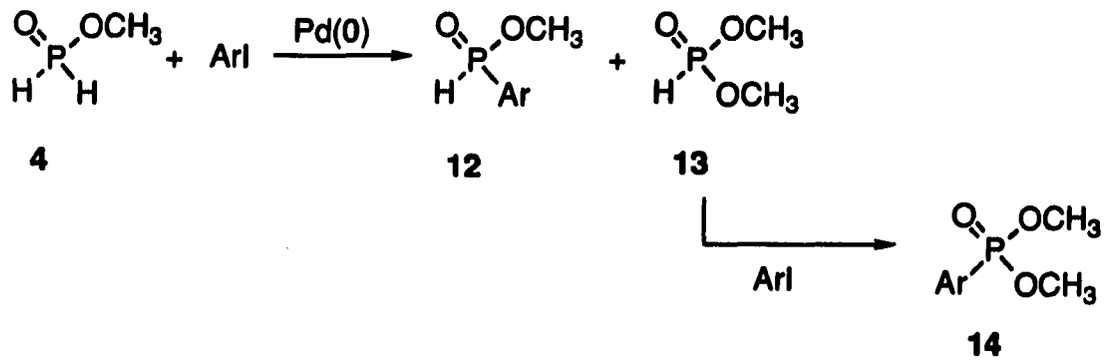
Method	Amount of 4	Reaction time	Yield of 11 (NMR integration)
a	5 eq.	1 h	54 %
b	5 eq.	1 h	65 %
c	3 eq.	1.5 h	24 %
d	5 eq.	1 h	trace
e	2.6 eq.	1 h	80 % (isolated)

As the decomposition of methyl phosphinate seemed to be a problem, we added it slowly to a refluxing reaction mixture. The idea was that reaction to form methyl tolylphosphinate would compete with methyl phosphinate decomposition. The methyl tolylphosphinate could then react with a second iodotoluene to yield the stable ditolylphosphinate. Slow addition would ensure that there was sufficient methyl phosphinate (difficult to measure out since an unknown amount of decomposition takes place), but not so much that the less stable monoarylphosphinic acid was the main product. To our surprise, the slow addition led to monotolylphosphinate. The coupling reaction at reflux must not be as fast as had been supposed, and the second coupling is clearly slower than the first one. Later, we found that the slow addition method did not help the reaction, but extended the reaction time because arylphosphinate formation is not a very rapid process. It actually took an hour in heated

CH_3CN solution. The reversed slow additions gave poor yields. Addition of methyl phosphinate to aryl iodide and catalyst solution rapidly by syringe seems to be the best way.

(4) The reaction yields are closely related to the amount of methyl phosphinate used. Symmetrical diarylphosphinate preparation requires two equivalents of aryl iodide per equivalent of methyl phosphinate. It has been found that when stoichiometric amounts of these starting materials were used, the aryl iodide was always left at the end of the reaction, indicating that methyl phosphinate partially decomposed before all aryl iodide was consumed. When 1.6 equivalents of aryl iodide were applied, the reaction went to completion and higher yield was obtained. We found that phosphinic acid was not measured accuratly volumetrically, so we always weighed it.

In the preparation of monophenylalanylphosphinates, we found that three equivalents of methyl phosphinate seem sufficient for the monoarylphosphinate formation. A large excess of methyl phosphinate inhibits the arylphosphinate reaction. It was observed that the reaction took long time to get started when 6 equivalents of methyl phosphinate 4 were used. An example is shown in Scheme 13 and Table 3. Once the reaction got started, it went to completion in about one hour, but the yield was poor. Long reaction time could cause the deiodination of aryl iodide.

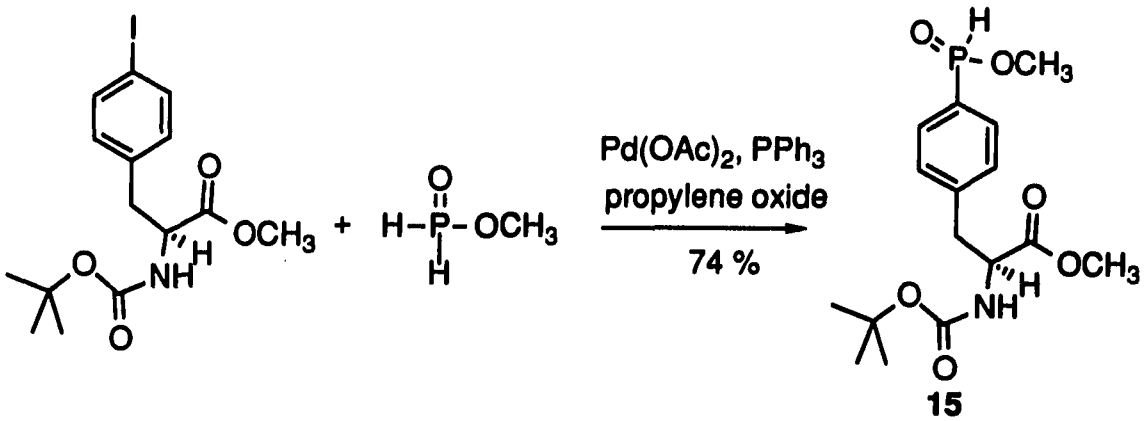


Scheme 12

Table 3. The effect of amount of methyl phosphinate on the reaction time

Amount of **4**	Reaction time (first step)	T °C (oil bath)	Yield of **15**
6 eq	2 days	85-90	47 %
3 eq	4 h	85-90	54 %

An excess of methyl phosphinate **4** also enhances the formation of arylphosphonate as a side-product, which has been isolated in our reactions. For example, 0.9% phosphonate was isolated from the preparation of **15.** Methyl arylphosphonates **14** have similar properties to the corresponding arylphosphinates in both TLC and ^{1}H NMR. The phosphonates **14** are difficult to separate from the phosphinates.



Scheme 13.

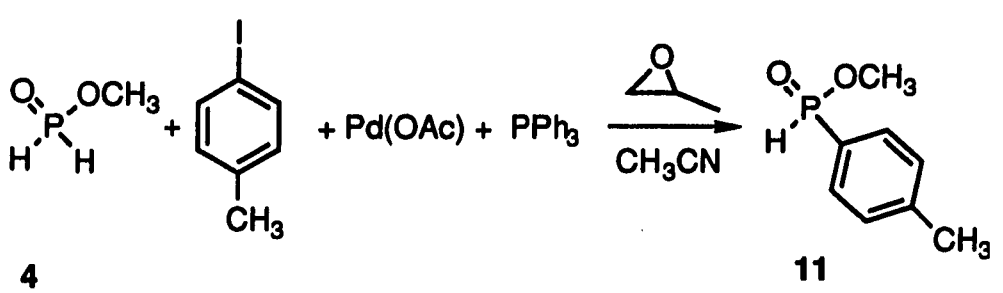
Dimethyl phosphonate **13** dissolves in aqueous $NaHCO_3$ solution so that it can be removed by basic wash. Dimethyl phosphonate **13** does not effectively compete with methyl phosphinate **4**, but it can interfere in the second step.

(5) Palladium acetate in the preance of 4 equivalents of PPh_3 was the first palladium catalyst used in our monoarylphosphinate reactions. Since the catalytic species in the reaction is palladium(0), tetrakis(triphenylphosphine)palladium appeared to be a reasonable choice. But we only tried this palladium(0) catalyst in the very early stage before finding the optimal reaction conditions. This palladium(0) catalyst worked similarly with palladium acetate. Palladium acetate was chosen as the catalyst in the synthesis of monoarylphosphinates because it is more stable than the palladium(0) catalyst. Tetrakis(triphenylphosphine)palladium is a good catalyst for the second step of making unsymmetrical diarylphosphinate. Later, palladium acetate has been replaced by a more stable preformed palladium complex, bis(triphenylphosphine)palladium chloride, which was

prepared from triphenylphosphine and palladium chloride through a simple procedure.[21]

This preformed palladium complex works as well as palladium acetate/PPh_3, but it slows down the reaction rate by a factor of 2 or 3. Its advantage is that it provides a more convenient single species to weigh out and somewhat more consistant results. Palladium chloride and nickel chloride have been tried in the monoarylphosphinate reactions. No arylphosphinate was formed in those reaction, but aryl iodide remained.

(6) A 4:1 ratio of triphenylphosphine to palladium acetate appears to be more effective than other ratio of phosphine to palladium. A series of reactions were run to determine this ratio. The reactions were carried out at 60°C for 3 hours. Since the reaction temperature was set lower than the normal temperature for this type of reactions, this series of reactions did not go to completion. From ^1H NMR spectra which were taken at one and three hours, we calculated the ratio of product to unreacted iodotoluene and summarized the results in Table 4.



4 11

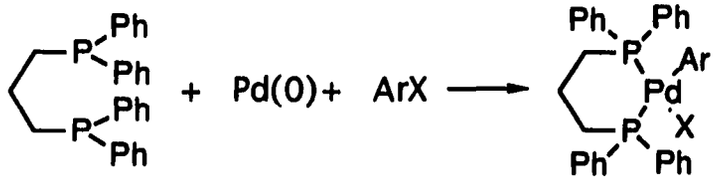
Scheme 14.

Table 4. The effect of ratio of PPh_3: Pd on the reaction yields

PPh_3/Pd	T °C	Reaction time (hours)	Ratio (NMR integration) 11/ArI
1:1	60	1	0.08
2:1	60	1	0.16
3:1	60	1	0.19
4:1	60	1	0.22
1:1	60	3	0.12
2:1	60	3	0.48
3:1	60	3	0.64
4:1	60	3	0.63

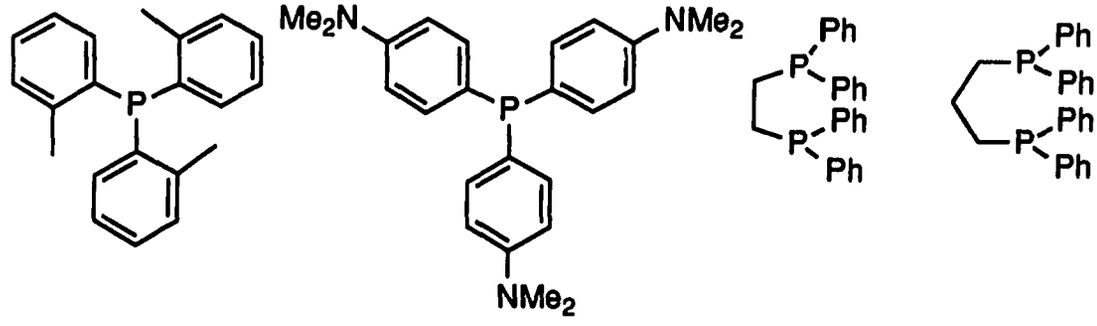
Table 4 showed that 4:1 ratio of triphenylphosphine to palladium gave higher yield of product than other combinations of catalyst and ligand.

Kruse et al.[22] and Milstein et al.[23] have demonstrated the rate enhancement by using 1,3-bis(diphenylphosphino)propane ligand in palladium catalyzed reactions (Scheme 15). They reported that the accelerations in rate could be a consequence of the obligatory *cis* arrangement of the ligands around palladium in the square planar complex, in contrast to the *trans* arrangement of the triphenylphosphine ligands in this complex.



Scheme 15.

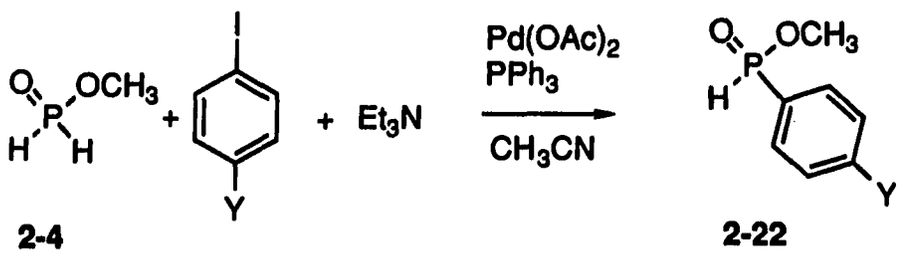
Besides triphenylphosphine, other ligands were also scanned, such as tritolylphosphine **18**, tris(4-dimethyl aminophenyl)phosphine **19**,1,2-bis(diphenylphosphino)ethane **20** and 1,3-bis(diphenylphosphino)propane **21**, in order to find out if any ligand can work at room temperature. The reactions iodotoluene with methyl phosphinate at room temperature gave only trace amount of tolylphosphinates with any of these catalysts. At 80°C, ligand **21** showed similar reactivity to triphenyl phosphine, and the other lugands gave worse results.



18 **19** **20** **21**

(7) A temperature dependence experiment of the reaction of methyl phosphinate with iodotoluene, carried out in deuterated acetonitrile and followed by ^1H NMR, showed that at room temperature or below 60°C the

arylphosphinate formation was very slow. At 70-82°C, the desired reaction proceeds rapidly and high yields of monoarylphosphinate product is obtained, even though the decomposition of methyl phosphinate is quite rapid at these temperatures (Table 5).



2-4 2-22

Scheme 16.

Table 5. The temperature effect on the arylphosphinate reaction

Amount of 4	Y	Reaction time & temperature	Yield of 22 (NMR integration)
6 eq.	CH_3	1 h at RT	trace
		1 h at 80 °C	79 %
7 eq.	H	1 h at RT	trace
		1 h at 48°C	12 %

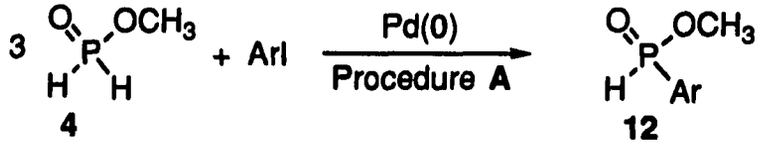
(8) Monoaryl and diarylphosphinates are usually purified by chromatography. Diarylphosphinates are quite stable to flash chromatography and can be purified easily with mixed eluant acetonitrile/CH_2Cl_2 or ethyl

acetate/hexanes. In contrast to diarylphosphinates, monoarylphosphinates decompose on silica gel. These decomposed products have been confirmed by a two dimensional TLC experiment since they migrate slowly on the silica TLC plate. This is the reason for the low yield of methyl monoarylphosphinates isolated in the more polar cases, such as 4-hydroxyphenyl phosphinate and 4-nitrophenylphosphinate. In the preparation of unsymmetrical diarylphosphinate, it is not necessary to separate the monoarylphosphinate. Excess methyl phosphinate and some byproducts can be removed by silica gel filtration and basic extraction. Since methyl phosphinate and dimethyl phosphonate dissolve in saturated $NaHCO_3$ solution nicely, the extraction method is more effective than filtration. If dimethyl phosphonate is not removed at this stage, it could further react with the second aryl iodide to form arylphosphonate which is very difficult to separate from the desired product.

(9) A large quantity of arylphosphinates can be readily prepared by the procedure we described above. Methyl *p*-phenylphenylphosphinate **12b** has been synthesized in 69% yield in a 1.5g scale.

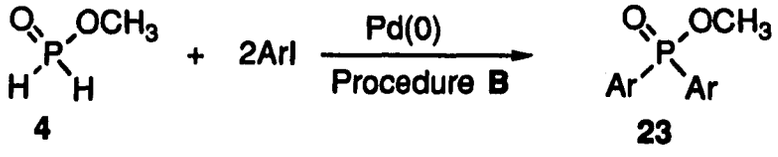
(10) Identification of arylphosphinate products is simplified by their characteristic ^1H NMR spectral signals. Methyl monoarylphosphinates may be recognized by P-H signals at d 7.4-7.1 (J_{PH} = 550-633 Hz). The protons of the methoxy bound to phosphorus demonstrate a 12 Hz coupling constant caused by phosphorus splitting in the monoaryl cases. This is in contrast to the 11 Hz coupling constant observed in the diaryl cases. Because of phosphorus coupling, many proton and carbon NMR signals are split.

Three optimized procedures have been developed for the synthesis of monoarylphosphinates, symmetrical diarylphosphinates and unsymmetrical diarylphosphinates respectively.[24] The procedure **A** conveniently converts aryl iodides to methyl monoarylphosphinates **12**. A solution of 3 equivalents of methyl phosphinate in trimethyl orthoformate was added to a mixture of the aryl iodide, palladium acetate (5mol% Pd based on aryl iodide), triphenylphosphine (or bis(triphenylphosphine)palladium dichloride) and tertiary amine in acetonitrile at room temperature. The monoarylphosphinate **12** was formed during the reflux and purified by chromatography.



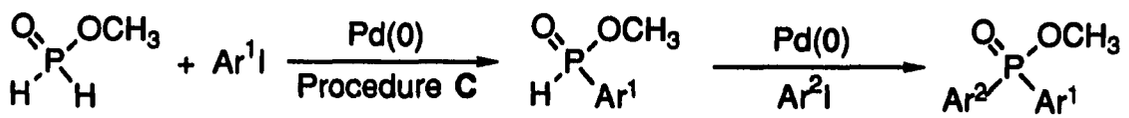
Scheme 17.

The procedure **B** provides symmetrical diarylphosphinate **23** merely by reducing the amount of methyl phosphinate to a nearly stoichiometric amount (0.5 -0.7 eq.) otherwise using in procedure **A** except that a longer reaction time is usually needed. This allowed further substitution of the first formed monoarylphosphinate. Diarylphosphinate products **23** are much more stable than monoaryl compounds.



Scheme 18.

Unsymmetrical diarylphosphinates **24** were prepared by procedure **C**. In the first part of procedure **C**, procedure **A** was followed to couple methyl phosphinate **4** with the first aryl iodide. The excess methyl phosphinate was removed by filtration through silica gel or basic aqueous extraction. The crude monoarylphosphinate **12** was allowed to react with the second aryl iodide under the catalysis of tetrakis(triphenylphosphine)palladium to form diarylphosphinate **24**. Pd(0) catalyst was used for this step to aviod wasting the intermediate as a reducing agent.



4 **12** **24**

Scheme 19.

Table 6 summarizes the arylphosphinates which were synthesized by the three procedures. We have made an important modification to these procedures. Tertiary amines are commonly used in most palladium catalyzed reactions. The reactions listed in Table 6 were carried out by using N-methylmorpholine or triethylamine as bases to neutralize HI formed during the reaction, and presumably to deprotonate the phosphinate to increase its reactivity.

Recently, we have noted that propylene oxide is a better HI scavenger than amines. Propylene oxide quenches HI sufficiently so that t-Boc amino protecting groups are not cleaved. In the tertiary amine case, ammonium salt precipitate causes a heterogeneous reaction mixture.

Table 6. Arylphosphinates prepared from aryl iodides and methyl phosphinate

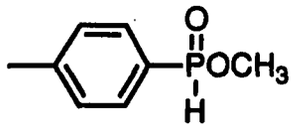
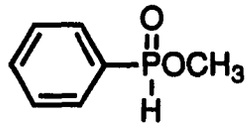
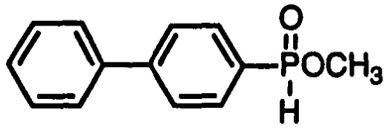
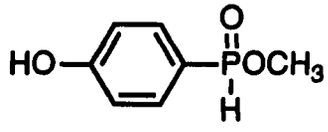
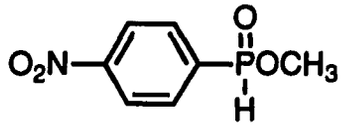
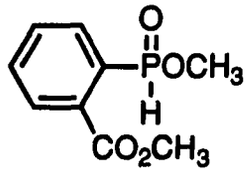
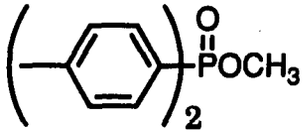
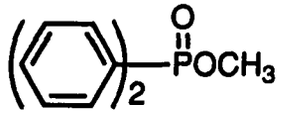
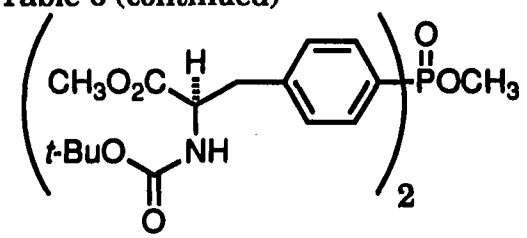
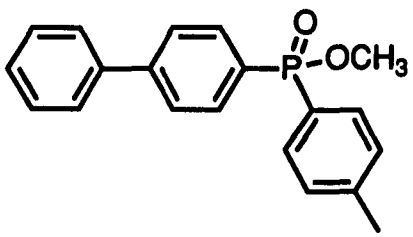
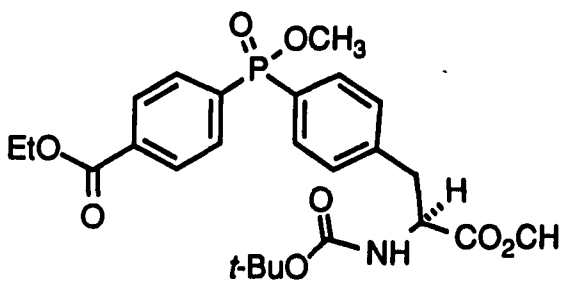
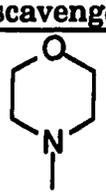
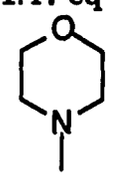
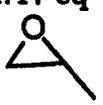
Product	Method	Isolated yield (%)	J_{PH} (Hz) of $POCH_3$ group
	A **11**	80	12.0
	A **12a**	63	12.0
	A **12b**	69	11.9
	A **12c**	42	12.1
	A **12d**	23 (NMR yield)	12.0
	A **12e**	44	12.3
	B **23a**	55	11.1
	B **23b**	49	11.1

Table 6 (continued)

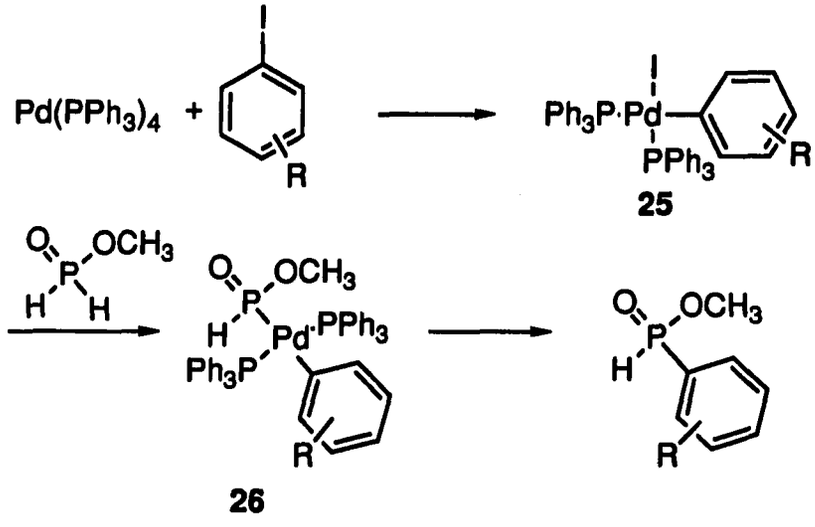
	B **23c**	59	11.2
	C **24a**	51	11.2
	C **24b**	44	11.2

The product of propylene oxide and HI is soluble in organic solvents. The reactions go faster in the homogeneous solution. The major operational advantage of this modification is that the need for anhydrous conditions is lessened because in the absence of base, any free phosphinic acid that forms by hydrolysis is reconverted to methyl phosphinate in situ. When 3 eq of methyl phosphinate and 3 eq of water reacted with iodotoluene in the presence of propylene oxide, 77% monotolylphosphinate was formed based on ^1H NMR integration. In the N-methylmorpholine case, when only 1 eq H_2O was in the reaction mixture, the yield of monotolylphosphinate dropped to 13% based on ^1H NMR integration. These results are in Table 7.

Table 7. The effect of water in thepreparation of methyl monotolylphosphinate

Amount of 4	HI scavenger	Amount of H_2O	T °C	Reaction time	Yield
2.6 eq	 1.47 eq	0	80	1 h	80 %
3 eq	 1.47 eq	1 eq	80	1 h	13 % (NMR Integration)
3 eq	 10 eq	3 eq	80	1.5 h	77 % (NMR integration)

This type of arylphosphinate formation may be rationalized by the oxidative addition and reductive elimination mechanism.[25] Pd(II), which is the original form of the palladium catalyst, is reduced to Pd(0) by a reductant in the reaction mixture which could be methyl phosphinate. An intermediate complex **25** of palladium and aryl iodide formed by oxidative insertion attacks methyl phosphinate to give intermediate **26**. The intermediate **26** then undergoes reductive elimination to give the arylphosphinate and regenerate the Pd(0) species.

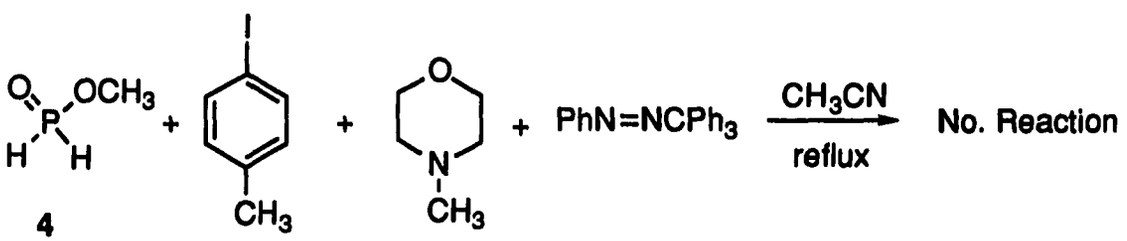


Scheme 20.

Recently, Curran et al. has reported that isomerization of unsaturated α-iodocarbonyls to cyclic g -iodocarbonyls gave identical product mixtures by using catalytic amount of either hexalkylditin or palladium (0).[26] They suggest that palladium serves a dual role as a radical initiator and as a trap of iodine. Aryl radicals are involved in cross-couplings of certain vinyl stannanes and aryl iodides,[27] a transformation which can also be catalyzed by

palladium.[21,25] Phosphonyl radicals are also capable of attacking aromatic rings.[28]

In order to distinguish organometallic and free radical chain reaction mechanisms involved in our arylphosphinate formation, we have run the reaction of methyl phosphinate **4** with iodotoluene in procedure **A** but replaced palladium acetate and triphenylphosphine with 0.01 eq. of phenylazotriphenylmethane, an initiator known to produce aryl radicals at 80°C (Scheme 21 and Table 8).



Scheme 21.

Table 8. Comparison of the ditolylphosphinate formation catalyzed by $Pd(OAc)_2$ or initiated by $PhN{=}NCPh_3$

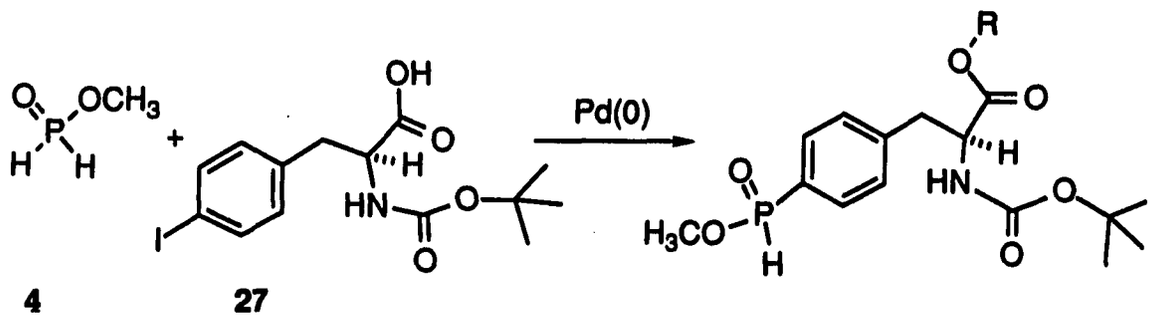
4	Aryl iodide	Catalyst or initiator	Base	Yield of **23b** (NMR integration)
0.56 eq.	Iodotoluene	$Pd(OAc)_2$	Et_3N	55 %
0.71 eq.	Iodotoluene	$PhN{=}NCPh_3$	Methylmorpholine	0 %

If the reaction underwent a free radical pathway, arylphosphinate might be formed through aryl or phosphinyl radical intermediates by initiation with phenylazotriphenylmethane. Since no aryl phosphinate was detected, a large amount of iodotoluene was left, but methyl phosphinate was consumed the free radical mechanism has been ruled out. Our very mildly basic conditions are in this way quite different from the $S_{RN}1$ conditions in Bunnett's report.[9]

Our method is compatible with quite a variety of functional groups, as can be seen in Table 6. In addition, nitriles, alcohols, and formate esters do not affect the reaction since acetonitrile, methanol and methyl formate are in the reaction mixture already. The poor yield of the *p*-iodonitrobenzene reactions may be due to partial reduction of the nitro group competing with the desired reaction. Khai and Arcelli have demonstrated that sodium hypophosphite hydrate is able to reduce nitrobenzene to aniline in the presence of a heterogeneous palladium catalyst.[29] We have not attempted to isolate these side products. Methyl *o*-methoxycarbonylphenylphosphinate was synthesized in reasonable yield (44%) from procedure **A**, but we failed to make di(o-methoxycarbonylphenyl)phosphinate, presumably because of the steric hindrance of the methoxycarbonyl group in the ortho position.

It has been tried to carry free carboxylic acid in the arylphosphinate formation. The product from the reaction of N-*t*-Boc-iodophenylalanine **27** with methyl phosphinate **4** did not look like mono N-*t*-Boc-phenylalanylphosphinate **28** by TLC and its solubility in base. The product could be an ester **29** because the acid group had a good chance to be esterified during the reaction. Hirao et al. reported the phosphonation of *o*-bromobenzoic acid and found it was subjected to esterification with diethyl phosphite as well

as phosphonation to give diethyl o-ethoxycarbonylphenylphosphonate in 24% yield.[10]



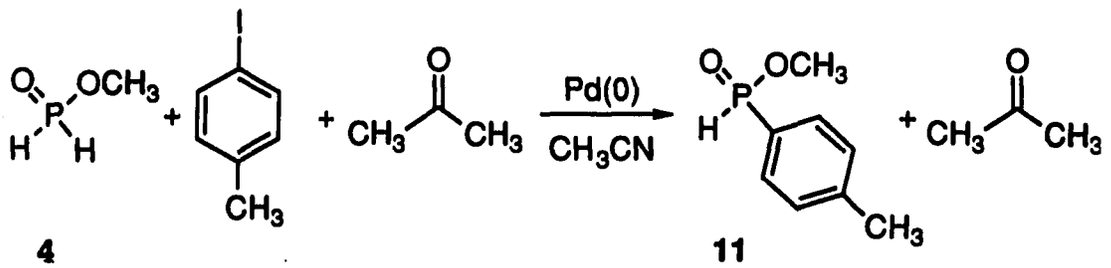
4 27

28 R = H
29 R = alkyl

Scheme 22.

Ketones may not be compatible with the reaction conditions described above as they readily react, both with methyl phosphinate 4 [19] and with product methyl arylphosphinates. However, when the monoarylphosphinate reaction was run in the presence of one equivalent of acetone based on aryl iodide, the ^{1}H NMR spectrum of the crude product appeared equivalent to that of reactions run in the absence of acetone (Scheme 23 and Table 9). It seems acetone did not affect the arylphosphinate formation. This aspect was not pursued further.

In most of the palladium-catalyzed processes reported in literature, aryl bromides and aryl triflates were used commonly as aryl group sources. Usually aryl bromides or triflates react similarly to, though more slowly than, iodides.



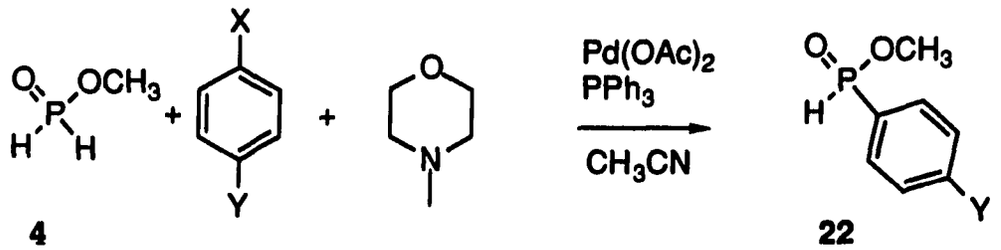
4 11

Scheme 23.

Table 9. The effect of acetone in the preparation of methyl monotolylphosphinate

Amount of **4**	Propylene oxide	Amount of acetone	Yield of **11** (NMR integration)
3 eq	10 eq	3 eq	100 %
3 eq	10 eq	0	77 %

In a rare exception, Andersson and Hallberg found that enol triflates were superior to vinyl iodides with respect to both reaction rate and selectivity in the vinylation of vinyl ethers.[30] *p*-Bromonitrobenzene, 4-toluene triflate, and N-*t*-Boc-tyrosine methyl ester triflate have been tried in the procedure **A** (Scheme 24 and Table 10). We were not able to obtain arylphosphinate products from these reactions. Presumably this is because the competitive decomposition of methyl phosphinate does not allow product formation, and not because of a fundamental difference in mechanism.



4 22

Scheme 24.

Table 10. Comparison of different aromatic nuleophiles in monoarylphosphinate reactions

Amount of 2-4	X	Y	T °C	Reaction time	Yield of **22** (NMR integration)
4.4	Br	NO_2	80	1 h	0
6.6	OTf	CH_3	80	2.5 h	0
1.1	OTf	CH_3	80	20 h	0
6.3	I	CH_3	80	2.5 h	62

When we first designed the connection between the phosphorus atom and amino acid to make building blocks for construction of binding sites, tyrosine was chosen to react with methyl phosphinate in the form of tyrosine triflate. Unfortunately this route did not work. Therefore we developed a new method for iodination of phenylalanine. This method provided iodophenylalanine as a perfect precursor to the arylphosphinate reactions.

It should be mentioned that some experiments tried at the early stage were not performed under the optimal conditions which were developed later. It could be worthwhile to rerun these experiments. In conclusion, we have developed a convenient route to a wide variety of phosphinates. This route is compatible with functionalities not stable in other procedures. we have synthesized building blocks for the binding site construction.

EXPERIMENTAL SECTION

General Procedure

^{1}H NMR spectra were obtained at 300 MHz on a Nicolet NT-300 or a Varian VXR-300 and ^{13}C NMR spectra at 75.43 MHz on the same instruments; chemical shifts are reported relative to TMS or DSS, couping constants (J) in Hz. ^{13}C NMR spectra are ^{1}H decoupled; the coupling constants reported are for doublets due to ^{31}P. ^{31}P NMR spectra were obtained at 121.42 MHz on a Varian VXR-300, chemical shifts are reported relative to external standard H_3PO_4. Mass spectra were obtained on a Kratos MS-50. Infrared spectra (IR) were measured with a IBM IR98 FTIR spectrophotometer. Flash chromatography was carried out as described.[31] CH_3CN used for reactions (not chromatography) was freshly distilled from P_4O_{10}. CH_2Cl_2, pyridine, THF, N-methyl morpholine and diisopropylethylamine used for reactions were freshly distilled from CaH_2. Other materials were used as received from Aldrich, except for drying in *vacuo*.. Glassware was predried at > 80°C. Kieselgel 60 silica gel (Merck) was used in flash chromatography. Kieselgel 60 F_{254} (Merck) TLC plates were used in thin layer chromatography. Some abbreviations used in this paper are defined as: MEK: methylethyl ketone, TFA: trifluoroacetic acid, HOAc: acetic acid, RT: room temperature, THF: tetrahydrofuran, EtOAc: ethyl acetate, sat'd.: saturated, dist: distilled.

Methyl-*p*-tolyl phosphinate 11[32] (procedure A)

A solution of methyl phosphinate in trimethylorthoformate is prepared by the method of Fitch[19]: Anhydrous crystalline phosphinic acid, prepared by rotary evaporation of 50% aqueous phosphinic (hypophosphorous) acid[19] (52 mg, 7.9×10^{-4} mol), is allowed to react with trimethylorthoformate (490 mg, 4.6×10^{-3} mol) in a pressure equalizing funnel at 23°C under N_2 for 1 h. This methyl phosphinate solution is added to a solution of *p*-iodotoluene (65.2 mg, 2.99×10^{-4} mol), N-methyl morpholine (30 mg, 3.0×10^{-4} mol), palladium acetate (3.4 mg, 1.5×10^{-5} mol), and triphenylphosphine (15.7 mg, 6.0×10^{-5} mol) in 1.5 ml of CH_3CN. This yellow solution is refluxed under N_2 for 1 h, cooled to room temperature, and the solvent removed at reduced pressure. The dark brown residue is triturated with ethyl acetate, the ethyl acetate filtered, and the solvent removed at reduced pressure. The resulting yellow oil is purified by flash chromatography (CH_3CN) to yield 40.8 mg (80%) methyl-*p*-tolyl phosphinate as a colorless oil. ^1H NMR ($CDCl_3$) δ 7.54 (d, J = 564.1, 1 H), 7.67 (dd, J = 8.0, 13.6, 2 H), 7.33 (dd, J = 3.1, 7.9, 2 H), 3.78 (d, J = 12.0,3 H), 2.43 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 143.89, 130.95 (J = 12.0), 129.49 (J = 14.2), 126.06 (J = 13.40), 51.48 (J = 7.1), 21.75; IR (neat) 2341, 1603, 1234, 959, 791 cm^{-1}; MS (M+) observed (calcd for $C_8H_{11}O_2P$) 170.04935 (170.04967).

Methyl phenylphosphinate 12a[5]

Procedure A is followed, starting with iodobenzene (61 mg, 2.99×10^{-4} mol). Flash chromatography (CH_3CN) yielded 29.3 mg (63%) methyl phenylphosphinate as a colorless oil. This material is identical chromatographically and spectroscopically to an authentic sample prepared by

treatment of phenyl dichlorophosphine with methanol.[5] ^{1}H NMR ($CDCl_3$) δ 7.57 (d, J = 565.4, 1 H), 7.80-7.52 (m, 5 H), 3.80 (d, J = 12.0, 3 H); IR (neat, cm^{-1}) 2351, 1593, 1231, 1042, 997, 798.

Methyl *p*-phenylphenyl phosphinate 12b

The propylene oxide modification of procedure A is followed; starting with 4-iodobiphenyl (1.48 g, 5.28 x 10^{-3} mol) and propylene oxide (3.70 ml, 5.3 x 10^{-3} mol) instead of N-methylmorphine. Flash chromatography (EtOAc) and recrystallization from EtOAc/hexane gives methyl *p*-phenylphenyl phosphinate (840 mg, 69%). mp 55-56°C; ^{1}H NMR ($CDCl_3$) δ 7.61 (d, J = 566.5, 1 H), 7.89-7.40 (m, 9 H), 3.83 (d, J = 11.9, 3 H); ^{13}C NMR ($CDCl_3$) δ 146.08, 139.73, 131.52 (J = 12.6), 128.98, 128.35, 127.85 (J = 133.0), 127.49 (J = 14.1), 127.31, 52.12 (J = 6.6); IR (neat, cm^{-1}) 2359, 1242, 1038, 962, 797; Mass m/z (M^+) calcd for $C_{13}H_{13}PO_2$ 232.06532, observed 232.06543.

Methyl *p*-hydroxyphenyl phosphinate 12c

Procedure A is followed, starting with p-iodophenol (82.3 mg, 3.74 x 10^{-4} mol). Flash chromatography (CH_3CN) gives 29.3 mg (46%) methyl p-hydroxyphenyl phosphinate as a colorless oil. This material is *ca.* 95% pure. Repeated chromatography gives less pure material as the desired product decomposes, but is not well separated from an impurity. ^{1}H NMR ($CDCl_3$) δ 9.50 (s, 1 H), 7.54 (d, J = 569.7, 1 H), 7.61 (dd, J = 8.5, 13.5, 2 H), 7.04 (dd, J = 2.8, 8.5, 2 H), 3.78 (d, J = 12.1, 3 H); ^{13}C NMR ($CDCl_3$) δ 162.41 (J = 3.0), 133.15 (J = 13.6), 116.41 (J = 15.2), 52.7 (J = 6.6); IR (neat, cm^{-1}) 3123, 2380,

1603, 1290, 1200, 1076, 962. Mass m/z ($M-H^+$) cald for $C_7H_8O_3P$ 171.02111, observed 171.02093.

Methyl *o*-methoxycarbonylphenyl phosphinate 12e

Procedure A is followed starting with methyl *o*-iodobenzoate (92.4 mg, 3.53×10^{-4} mol). Crude product is purified by flash chromatography (4:1 CH_3CN/CH_2Cl_2) to yield 37.2 mg *o*-methoxycarbonylphenyl phosphinate as a colorless oil (46%). ^1H NMR ($CDCl_3$) δ 8.27-7.64 (m, 4 H), 8.04 (d, J = 616.8, 1 H), 3.97 (s, 3 H), 3.84 (d, J = 12.3, 3 H); ^{13}C ($CDCl_3$) δ 166.63, 133.76 (J = 7.6), 132.70 (J = 2.6), 132,53 (J = 12.1), 130.90 (J = 9.1), 130.58 (J = 14.6), 53.11 (J = 6.0), 52.73; IR (neat, cm^{-1}) 2407, 1726, 1223, 1038,1007, 797.

4,4''-(methoxyphosphinylidene)bis[N-[(1,1-dimethylethoxy) carbonyl]-L-Phenylalanine dimethyl ester 23c (procedure B)

Phosphinic acid (9.1 mg, 1.38×10^{-4} mol) and trimethylorthoformate (73 mg, 6.88×10^{-4} mol) are allowed to stand under N_2 for 1 h to form methyl phosphinate as described for procedure A.

(S)-N-*t*-BOC-*p*-iodophenylalanine methyl ester (79.7 mg, 1.9×10^{-4} mol), triphenylphosphine (4.0 mg, 1.52×10^{-5} mol), palladium acetate (9 mg, 3.8×10^{-6} mol), triethylamine (20 mg, 2.0×10^{-4} mol) are placed and sealed under N_2 in a 3 ml vial containing 450 ml of CH_3CN freshly distilled from P_4O_{10}. Methyl phosphinate solution is added by syringe. The yellow reaction mixture is then heated in an oil bath at 77 °C for 4 h. Solvent is removed from the dark brown solution at reduced pressure, and the residue was purified by flash chromatography (ethyl acetate) to yield 35.6 mg product (59%). ^1H NMR

($CDCl_3$) δ 7.73 (dd, J = 8.1, 12.0, 4 H), 7.24 (dd, J = 22.7, 8.1, 4 H) , 5.02-5.00 (m, 2 H), 4.61-4.59 (m, 2 H), 3.74 (d, J = 11.1, 3 H), 3.71 (s, 6 H), 3.22-3.00 (m, 4 H), 1,40 (s, 18 H); ^{13}C NMR ($CDCl_3$) δ 171.85, 154.91, 140.85, 131.83 (J = 8.2), 129.58 (J = 13.5), 129.55 (J = 137.51), 80.08, 54.14, 52.35, 51.52 (J = 6.8), 38.39, 28.26; IR (neat, cm^{-1}) 3369, 1745, 1713,1221, 1020; mass m/z (M^+) calcd for $C_{31}H_{43}O_{10}N_2P$ 634.26554, observed 634.26649.

Methyl bis(*p*-methylphenyl)phosphinate 23a[33]

A modified procedure B is followed, starting with phosphinic acid (5.2 mg, 8.97×10^{-4} mol), *p*-iodotoluene (391.2 mg, 1.79×10^{-3} mol), triphenylphosphine (16.9 mg, 6.44×10^{-5} mol), palladium acetate (3.6 mg, 1.61×10^{-5} mol) and triethylamine (163 mg, 1.61×10^{-3} mol) using the glassware described in procedure A. After 1 hour of reflux, the solvent is removed at reduced pressure. The residue is dissolved in ethyl acetate, washed with sat'd. NaCl, and dried with $MgSO_4$. After removal of solvent at reduced pressure, crude product is purified by flash chromatography (85:5 ethyl acetate:hexane) to yield 25.8 mg product (55%).[33] ^1H NMR ($CDCl_3$) δ 7.69 (dd, J = 8.1, 12.3, 4 H), 7.25 (dd, J = 3.6, 8.1, 4 H), 3.73 (d, J = 11.1, 3 H), 2.37 (s, 6 H); ^{13}C NMR ($CDCl_3$) δ 142.55 (J = 2.5), 131.57 (J = 10.6), 129.20 (J = 13.7), 127.93 (J = 139.5), 51.30 (J = 6.0), 21.54 (J = 1.1); IR (neat, cm^{-1}) 1605, 1231, 1036, 928, 810.

Methyl diphenylphosphinate 23b

Procedure B is followed, starting with iodobenzene (204 mg, 1.00×10^{-3} mol). Product is purified by flash chromatography (4:1 ethyl acetate: hexane)

to yield 56.8 mg (49%) product as a white crystalline material, identical by chromatographic and spectral properties to an authentic sample prepared from diphenylphosphinyl chloride and methanol. ^{1}H NMR ($CDCl_3$) δ 7.83-7.45 (m, 10 H), 3.77 (d, J = 11.1, 3 H); ^{13}C NMR ($CDCl_3$) δ 132.13, 131.58 (J = 10.0), 130.95 (J = 138.2), 128.46 (J = 13.2), 51.48 (J = 6.5); IR (neat, cm^{-1}) 1591, 1437, 1229, 1024, 797.

Methyl *p*-methylphenyl-*p*-phenylphenyl phosphinate 24a (procedure C)

Procedure A is followed, using *p*-iodobiphenyl (84 mg, 3.0×10^{-4} mol), except that the monoaryl phosphinate is not isolated, the reaction mixture is filtered through silica gel to remove methyl phosphinate and its decomposition products, and volatile material removed at reduced pressure. This crude product is treated with tetrakis(triphenylphosphine)palladium (10.4 mg, 9×10^{-6} mol), *p*-iodotoluene (65.4 mg, 3×10^{-4} mol) and N-methyl morpholine (30 ml, 3×10^{-4} mol) in 2 ml freshly distilled CH_3CN. The resulting solution is refluxed for 2 h under N_2. The yellow reaction mixture is evaporated and purified by flash chromatography (8:3 ethyl acetate:hexane) to yield 49.3 mg of product (51%). This material is identical to that prepared by the reverse order of reaction (first iodotoluene, then iodobiphenyl) with isolation of the intermediate monoarylphosphinate. Yield over those two steps: 50%. ^{1}H NMR ($CDCl_3$) δ 7.90-7.27 (m, 13 H), 3.78 (d, J = 11.2, 3 H), 2.39 (s, 3 H); ^{13}C NMR ($CDCl_3$) δ 144.85 (d, J = 3.1), 142.81 (J = 3.9), 139.95, 132.09 (J = 10.1), 131.72 (J = 10.6), 129.94 (J = 139.0), 129.36 (J = 13.7), 128.90, 128.10, 127.76 (J = 140), 127.24, 127.21 (J = 13.1), 51.48 (J = 6.1), 21.63 (J = 1.0); IR (neat, cm^{-1})

1601, 1229, 1032, 791; mass m/z (M^+) calc'd for $C_{20}H_{19}PO_2$ 322.11227, observed 322.11163. Analysis: found (calcd): %C 74.69 (74.51), %H 5.99 (5.90).

N-[(1,1-dimethylethoxy) carbonyl]-4-[[4-(ethoxycarbonyl)phenyl] methoxyphosphinyl]-L-Phenylalanine methyl ester 24b

Procedure C is followed starting with (S)-N-*t*-BOC-*p*-iodophenylalanine methyl ester (125.3 mg, 2.99 x 10^{-4} mol) with a 2 h reflux in step one. The crude monoarylphosphinate in 2 ml CH_3CN is treated with ethyl 4-iodobenzoate (82.8 mg, 3 x 10^{-4} mol), and other reagents as described. Flash chromatography (ethyl acetate) gave 66.3 mg product (44%) as a colorless oil. ^1H NMR ($CDCl_3$) δ 8.11 (dd, J = 3.3, 8.3, 2 H), 7.88 (dd, J = 8.2, 11.8, 2 H), 7.74 (dd, J = 8.0, 12.1, 2 H), 7.26 (dd, J = 5.2, 11.9, 2 H), 5.07-5.04 (m, 2 H), 4.61-4.58 (m, 1 H), 4.39 (q, J = 7.1, 2 H), 3.78 (d, J = 11.2, 3 H), 3.71 (s, 3 H), 3.17-3.06 (m, 2 H), 1.39 (t, J = 7.0, 3 H), 1.38 (s, 9 H); ^{13}C NMR ($CDCl_3$) d 171.89, 165.71, 154.95, 141.30 (J = 2.0), 135.73 (J = 134.9), 133.78 (J = 2.5), 131.88 (J = 10.1), 131.67 (J = 10.1), 129.86 (J = 138.0), 129.78 (J = 13.6), 129.52 (J = 13.1), 80.13, 61.46, 54.14, 52.40, 51.74 (J = 6.0), 38.44, 28.24, 14.28; IR (neat, cm^{-1}) 3267, 2980, 1720 (b), 1605, 1223, 1034, 798; mass m/z (M^+) calcd for $C_{25}H_{32}O_8NP$ 505.18656, observed 505.18654.

Methyl bis(*p*-nitrophenyl)phosphinate 24c

Procedure A is followed starting with *p*-iodonitrobenzene (93.1 mg, 3.74 x 10^{-4} mol). Product **12d** was formed in 23% yield. Yield is calculated on the basis of NMR integration (internal standard: 1,3,5-trimethoxybenzene). This product **12d** decomposed on silica gel and was not isolated in pure form. ^1H

NMR ($CDCl_3$) δ 8.38 (dd, J = 2.4, 8.7, 2 H), 8.01 (dd, J = 8.7, 12.8, 2 H), 7.65 (d, J = 577.5, 1 H), 3.87 (d, J = 12.0, 3 H); IR (neat, cm^{-1}) 2381, 1601, 1524, 1350, 1231, 1030, 995, 856, 797. Procedure B is followed, starting with *p*-nitro iodobenzene (335 mg, 1.35×10^{-3} mol). The product **24c** was purified by flash chromatography (ethyl acetate:hexanes 4:1). Purified product 11.4 mg was obtained as colorless oil (5%). ^1H NMR ($CDCl_3$) δ 8.34 (dd, J = 2.7, 9.0, 4 H), 8.03 (dd, J = 9.0, 11.4, 4 H), 3.88 (d, J = 11.1, 3 H); ^{13}C NMR ($CDCl_3$) δ 145.13, 137.01 (J = 136.0), 133.06 (J = 11.1), 123.88 (J = 13.6), 52.46 (J = 6.0); IR (NaCl, cm^{-1}) 1524, 1350, 1236, 1034, 850, 800; mass m/z (M^+) calcd for $C_{13}H_{11}N_2O_6P$ 322.03548, observed 322.03482.

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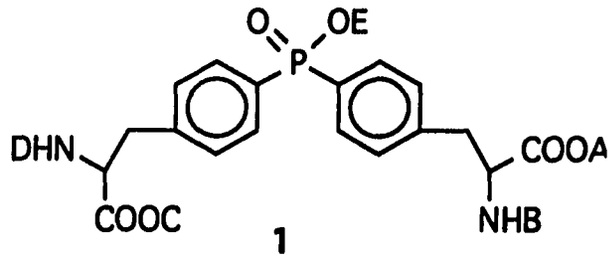
PAPER II. ASSEMBLY PROCEDURES USING BISPHENYLALANYLPHOSPHINATES

INTRODUCTION

A class of potential binding sites, as described in GENERAL INTRODUCTION, can be generated based on the building block bisphenylalanylphosphinate 1.[1] A significant feature of these arylphosphinates which we were going to make is the ease with which derivatives may be assembled, bearing variously sized and shape aromatic walls. Compound 1 is anovel bis amino acid in its protecte form which possesses a water solubility providing group, two aromatic rings which can build a hydrophobic wall and several functional groups available for further connection with other groups. Following is the discussion of the construction of this specific kind of building blocks and of methods for assembly of this kind of building blocks.



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A,B,C,D,E = protecting groups

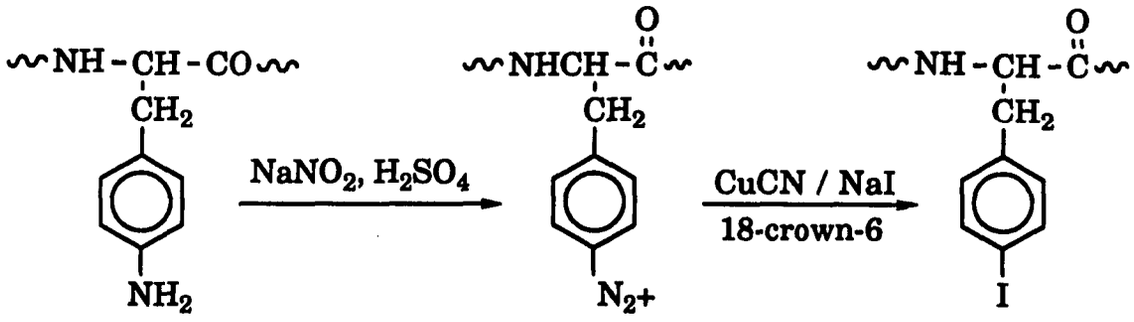
RESULTS AND DISCUSSION

Iodination of L-phenylalanine

The two main parts of the building block **1** are two protected phenylalanines. The key question is how to connect the phosphorus to the *para* positions of the phenylalanine. Tyrosine is a better candidate than phenylalanine because it is easy to convert the hydroxyl group to other groups. We have made tyrosine triflate and submitted it to the methyl arylphosphinate reaction which has been described in PAPER I. Unfortunately, the aryl triflate is not as reactive as an aryl iodo under the reaction conditions. Therefore we chose 4-iodo-L-phenylalanine as a precursor for making building block **1**.

4-Iodo-L-phenylalanine is commercially available, but very expensive (about $80/g). A large amount of iodophenylalanine is needed in our research project because it is the starting material in a multistep synthesis. We decided to prepare iodophenylalanine. One of the best known methods for the introduction of iodine into aromatic rings is the reaction of a diazonium salt with iodide ion.[2] Abderhalden first reported the synthesis of 4-iodophenylalanine from phenylalanine in 1909.[3] In his procedure, phenylalanine was first converted to 4-nitrophenylalanine which underwent reduction to give 4-aminophenylalanine. 4-Aminophenylalanine was treated with nitrous acid, followed by potassium iodide to give iodophenylalanine.

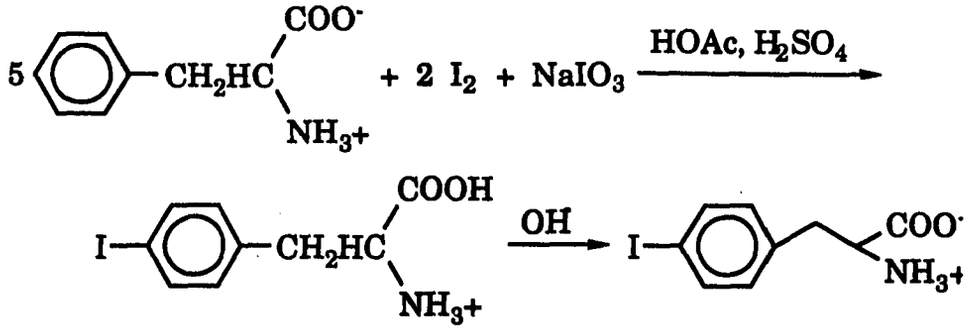
This route has been optimized for radioiodination of peptides,[4] as in Hruby and coworkers' procedure[5] in Scheme 1.



Scheme 1.

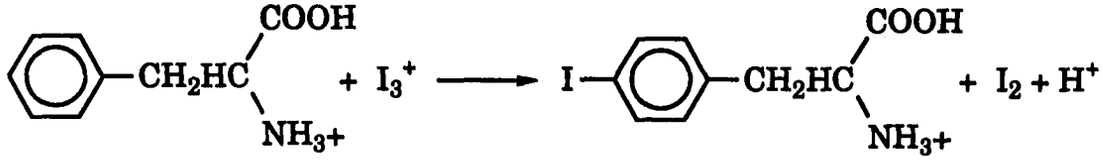
From a practical standpoint, the synthesis of iodophenylalanine should be straightforward, conveniently involving relatively simple reagents and high yield. The indirect route via diazonium was not appealing.[3] Iodination of aromatic compounds via electrophilic substitution is a well known method. Even aromatic rings bearing deactivating substituents can be directly iodinated.[6] Iodine is the least reactive of the halogens in aromatic substitution. Except for reactions with active substrates, an oxidizing agent must normally be present to oxidize I_2 to a better electrophile. When SO_3 or HIO_3 is the oxidizing agent, the oxidized iodine I_3^+ is the actual attacking species in the electrophilic substitution reaction.[7]

The synthetic pathway for making iodophenylalanine we chose is the direct electrophilic iodination of L-phenylalanine (Scheme 2). L-phenylalanine was dissolved in acetic acid and treated with iodine and sodium iodate in the presence of sulfuric acid.



Scheme 2.

In this reaction, the electrophilic attacking species was assumed to be I_3^+ which was generated by oxidation of I_2 with $NaIO_3$ in the acidic solution.



Scheme 3.

L-phenylalanine was dissolved in acetic acid and sulfuric acid first. The protonated carboxylic group is stable to oxidizing reagents. Iodine and sodium iodate were added to this acidic mixture. The dark purple mixture was refluxed for about one hour. At this point, most of the iodine was consumed and the reaction mixture was light purple. A little bit less than stoichiometric amount of iodine and a little bit more than stoichiometric amount of sodium iodate were used to avoid dark iodine color left at the end of the reaction. Sodium periodate has been also used in this reaction, which played a similar role to sodium iodate, but is more expensive. A longer reaction time did not

improve the product yield. The reaction was easily followed by TLC which distinguishes iodophenylalanine and phenylalanine very nicely. When the reaction was complete, the acetic acid was removed by rotary evaporation, the residue was diluted with water and extracted with methylene chloride to remove excess iodine. After this organic wash the brown acidic aqueous mixture decolorized with norit. The clear aqueous solution was neutralized with NH_3 to precipitate crude iodophenylalanine. The crude product was recrystallized from hot acetic acid twice to yield pure 4-iodo-L-phenylalanine in about 50% yield. The optical purity was checked by chiral TLC.[8] Racemic product was not seen.

It has been found that the yield and purity of product from this iodination reaction are closely related to the work up and purification procedures. A reaction mixture was divided into three portions. Different work up procedures were applied to each portion. Three procedures have been compared.

(a) Since the final product is recrystallized from acetic acid, the removal of acetic acid by evaporation after the reflux may not be necessary. It may be possible to only remove sulfuric acid and then to crystallize the product from acetic acid solution directly without the neutralization step. Sulfuric acid was converted to its sodium salt by adding NaOAc. Sodium sulfate precipitate was filtered out. The acetic acid filtrate containing the crude product was concentrated. Iodophenylalanine was crystallized from acetic acid. This procedure is simple and short, but the product yield was low (about 10%).

(b) When the reaction was completed, acetic acid was removed by evaporation. The residue was dissolved in H_2O and the product was

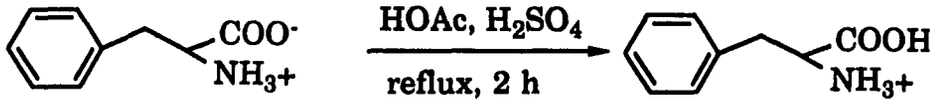
precipitated out when the solution was neutralized with ammonium hydroxide. This product was purified by recrystallization from acetic acid and H_2O mixture. The purified product was obtained in 36% yield.

(c) This procedure is similar to (b), but the precipitation step was repeated twice. The product was finally recrystallized from pure acetic acid. The yield of purified product was 53%.

This comparison indicates that the neutral precipitation is an effective step of separating iodophenylalanine and side products. NaOH and NH_4OH have been used to neutralize the acidic solution. Na_2SO_4 is only slightly soluble in cold water, but the solubility of $(NH_4)_2SO_4$ in cold water is about 70 g/100 ml. Obviously, NH_4OH is a better base to use for separation of iodophenylalanine and sulfate salt.

The recrystallization from acetic acid and H_2O did not give pure iodophenylalanine. Acetic acid and H_2O have been used in ratio of 7:3, 1:1 and 2:3. Only yellow crystal was obtained which gave low melting point. Pure acetic acid is a much better recrystallization solvent than the mixture. The recrystallized iodophenylalanine obtained from acetic acid is a white shiny crystal and has melting point above 250 °C.

We also tested the stability of phenylalanine in hot acetic acid and sulfuric acid (Scheme 4). After refluxing in acetic acid and sulfuric acid mixture for two hours, TLC and ^1H NMR showed that decomposed or racemized product was not formed.



Scheme 4.

Every step of this workup procedure was followed by HPLC analysis to see whether the yield could be improved beyond 50%. HPLC results showed that the percentage of the product lost during the work up and purification steps is very small. Since the reaction yield was only about 50%, half of phenylalanine must be converted to something else. In the reaction mixture, sometimes a trace of *ortho*-iodophenylalanine was detected. A large amount of unreacted phenylalanine or other side products have not been seen. We are not sure what becomes of the other half of the starting phenylalanine.

The synthetic method of iodination of L-phenylalanine is simple and suitable for our research purpose. A large amount of 4-iodo-L-phenylalanine has been made in reasonable yield and high purity. The cost is much lower than buying commercial iodophenylalanine. Furthermore, this procedure has been extended to the undergraduate organic chemistry teaching program. Chemistry students have run this reaction in the laboratory successfully and obtained the desired product, iodophenylalanine.

Protection of 4-Iodo-L-phenylalanine

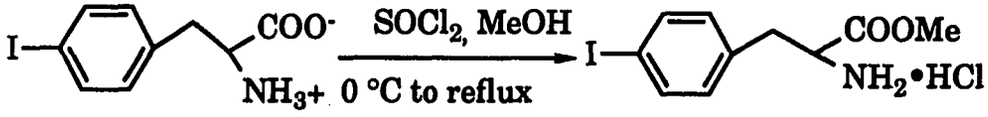
The chemistry of protecting groups is an important field of organic chemistry and peptide chemistry. Clearly, a successful peptide synthesis demands ordered sequential coupling of amino acids, with little byproduct formation. Such is achieved by the use of protecting groups for amino

functions and carboxyl functions of amino acids. Once the peptide bond is synthesized it becomes necessary to remove the protecting groups under conditions that will not affect the product. In our research, the iodophenylalanine protection is not only used for peptide bond formation, but also for the arylphosphinate reactions. One of the desired building blocks is the bisphenylalanylphosphinate with five different protecting groups which can be removed selectively under different conditions. Solubility is another reason to protect iodophenylalanine because the free amino acid is only soluble in acidic or basic aqueous solution, but not in organic solvents. Several protecting groups have been applied to iodophenylalanine.

Carboxyl protecting groups

Generally, carboxyl protecting groups are introduced by esterification. Four iodophenylalanine esters have been made.

Methyl ester The method we used to make iodophenylalanine methyl ester is the addition of the amino acid to a cold mixture of thionyl chloride and methanol.[9] Crude product was purified by recrystallization from methanol and ether. Iodophenylalanine methyl ester hydrochloride salt was obtained in 92% yield (Scheme 5).



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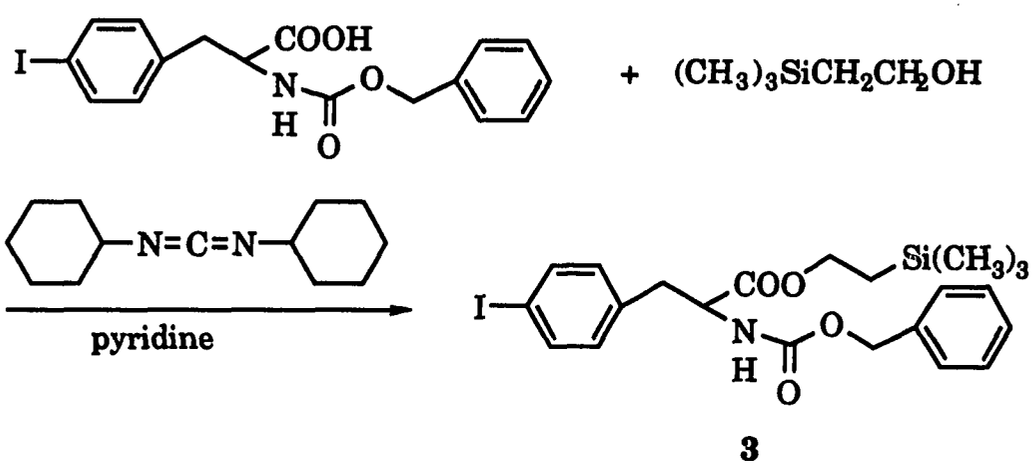
Scheme 5.

2-Trimethylsilylethyl ester N-Cbz iodophenylalanine trimethylsilylethyl ester **3** has been synthesized in 88% yield by the reaction of N-protected iodophenylalanine with 2-trimethylsilylethanol and dicyclohexylcarbodiimide (DCC) in the presence of pyridine (Scheme 6).

The most important feature of this ester is that the trimethylsilylethyl group is selectively removable with fluoride ion. No evidence of racemization by fluoride was found.[10]



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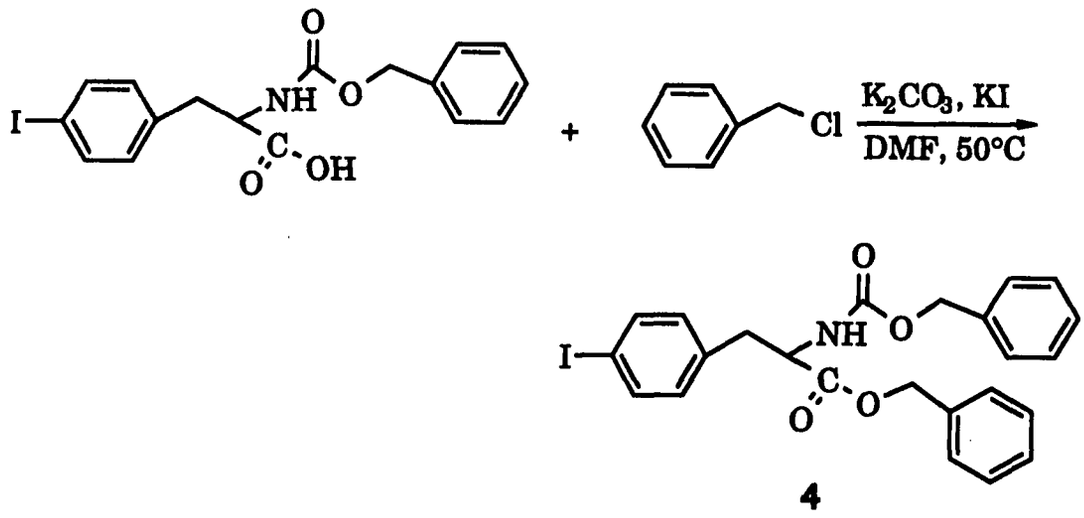
Scheme 6.

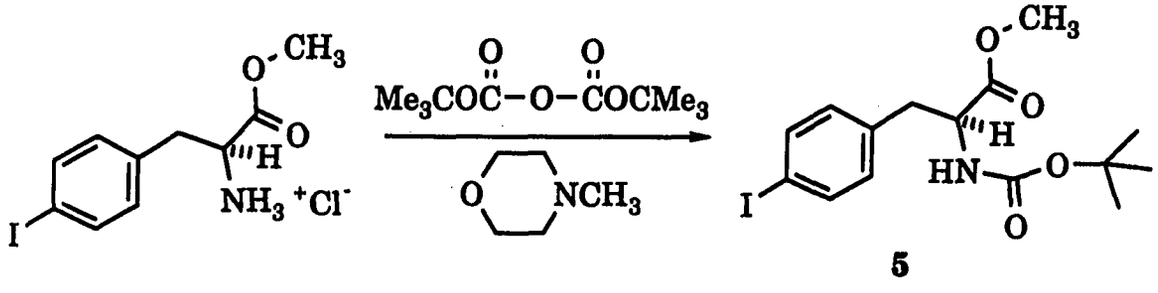
Benzyl ester We made the iodophenylalanine benzyl ester by the reaction of N-protected iodophenylalanine with benzyl chloride and potassium iodide in DMF at 50 °C (Scheme7).[11] The product **4** was obtained from amino and carboxyl two steps protection in 58%.



Scheme 7.

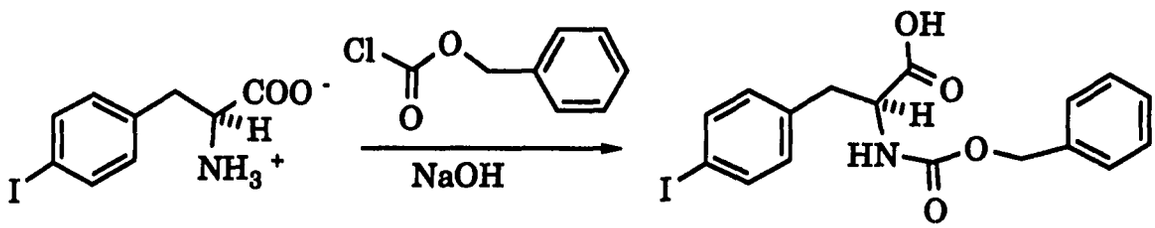
Amino protecting groups

***tert*-Butoxycarbonyl (*t*-Boc) group** The*t*-Boc group was introduced by the reaction of iodophenylalanine methyl ester and di-*tert*-butyl dicarbonate in the presence of N-methylmorpholine in CH_2Cl_2 (Scheme 8). Crude product was purified by flash chromatography or recrystallization. N-*t*-Boc iodophenylalanine methyl ester **5** was obtained from this reaction in 77% yield.



Scheme 8.

Benzyloxycarbonyl (Cbz) group Cbz protection is accomplished by the reaction of iodophenylalanine and benzylchloroformate in alkaline aqueous solution (Scheme 9). The N-Cbz-iodophenylalanine **6** was purified by recrystallization and obtained in 91% yield.



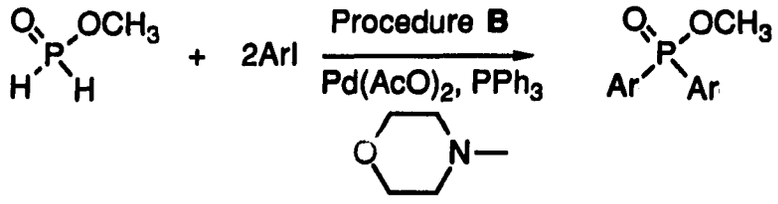
6

Scheme 9.

From the procedures described above, iodophenylalanine is readily protected in various ways and also can be deprotected selectively. It gives the flexibility we needed in the construction of binding sites.

Synthesis of Methyl Bisphenylalanylphosphinates

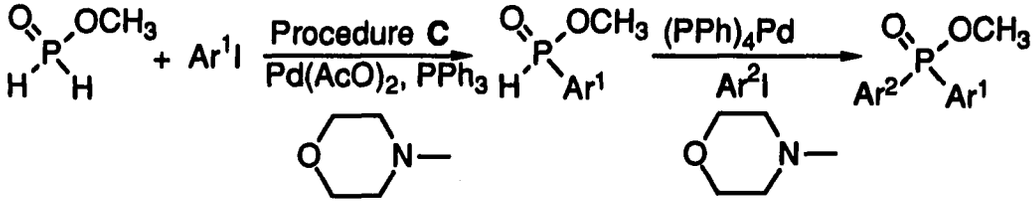
Methyl bisphenylalanylphosphinates are the building blocks for our binding sites. Several phosphinates have been prepared and used as building blocks. The synthesis of bisphenylalanylphosphinates developed for this purpose has been described in PAPER I of this thesis. Procedure **B** was used to synthesize symmetrical bisphenylalanylphosphinate **7** and **8**.



Scheme 10a.

In both compounds **7** and **8**, one protecting group can be removed without affecting the other one. Once all the protecting groups are removed by boiling in concentrated HCl, the free bis amino acid is formed.

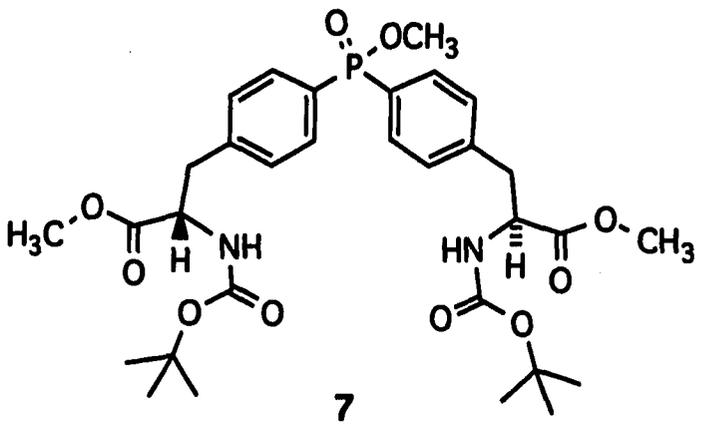
Compound **9** and **10** are unsymmetrically substituted bisphenylalanylphosphinates with five different protecting groups. Procedure **C** was employed in the preparation of these two compounds.



Scheme 10b.

For compound **9**, each protecting group can be removed without attacking the other three. This molecule which possesses such diverse functionality was prepared in a 5 step synthetic procedure. Differential protection alone of an unprotected bis amino acid would require more steps than our synthetic route to protected amino acid. The intermediate of **9**, monophenylalanylphosphinate, can be isolated by chromatography. This intermediate itself is an interesting compound. Its deprotected form showed

activity in tyrosine phosphatase inhibition. This compound is designed for preparing a macrocyclic peptide which is made by connecting two molecules of 9 with two peptide bonds.



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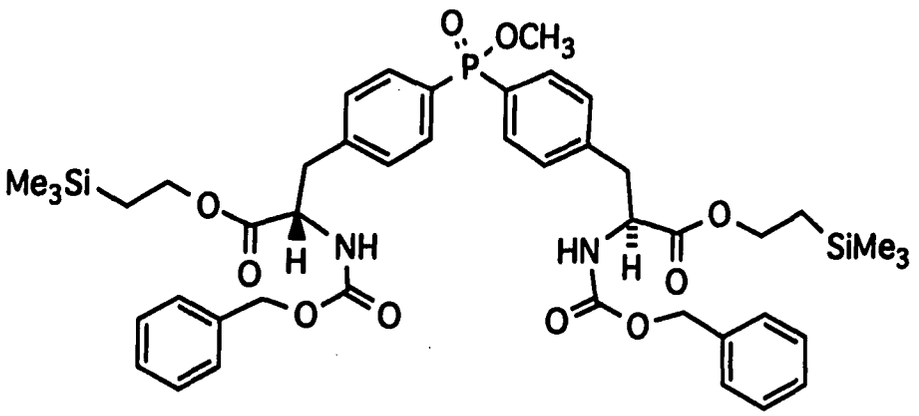

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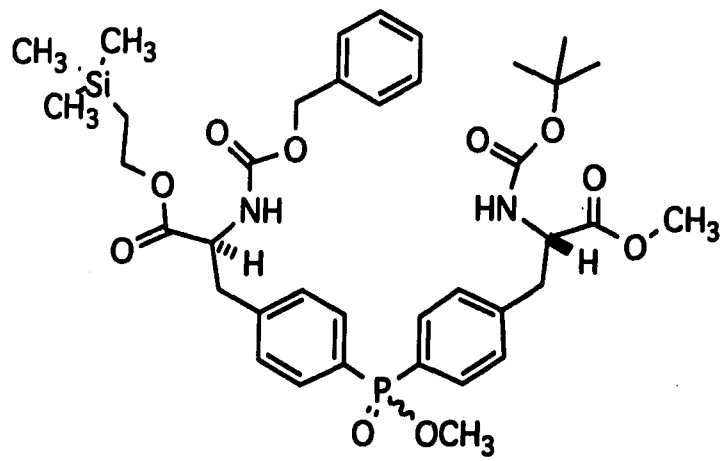

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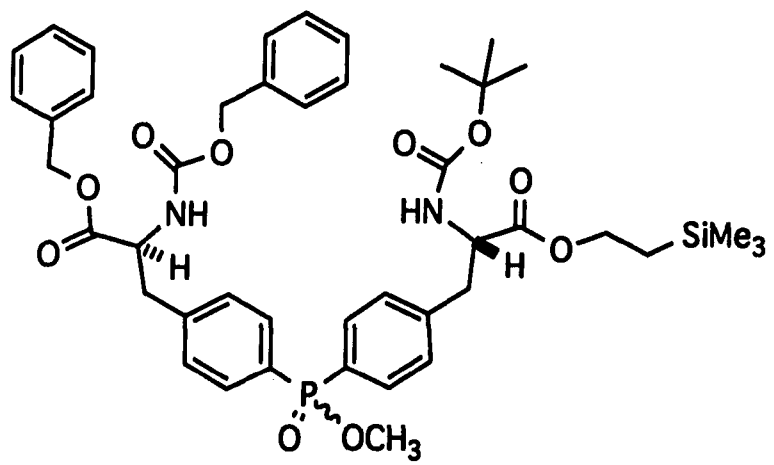


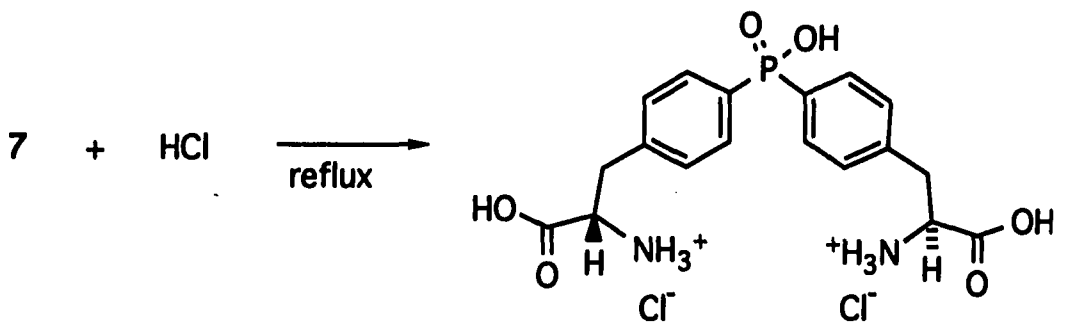
10

Assembly Procedures Using Bisphenylalanylphosphinates

Once bisphenylalanylphosphinates have been synthesized, the next step is to assemble these building blocks into our designed binding sites. We have developed procedures for this assembly.

4,4'-Hydroxyphosphinylidine bis phenylalanine and metal complexes

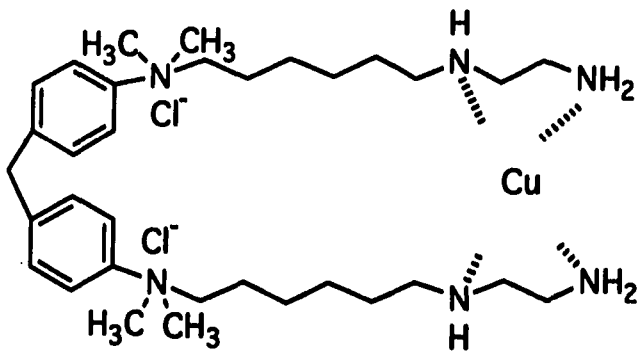
A preliminary investigation of the properties of the bis amino acid, 4,4'-hydroxyphosphinylidine bis phenylalanine **11** was made. This bis amino acid was obtained by refluxing phosphinate **7** in conc. HCl to remove all the protecting groups (Scheme 11). The completely deprotected bis amino acid **11** is very water soluble.



11

Scheme 11.

It is well known that amino acids coordinate to metal ions through N and O and the shapes and sizes of the resulting complexes depend on the metal ion. For example, glycine and Cu^{2+} form a very stable complex Cu-Gly_2.[12] Schneider and coworkers have demonstrated a macrocyclic binding site **12** cyclized by metallic complexation.[13] Several groups have made hydrophobic or other binding sites which are organized by a metal exist.[14,15]



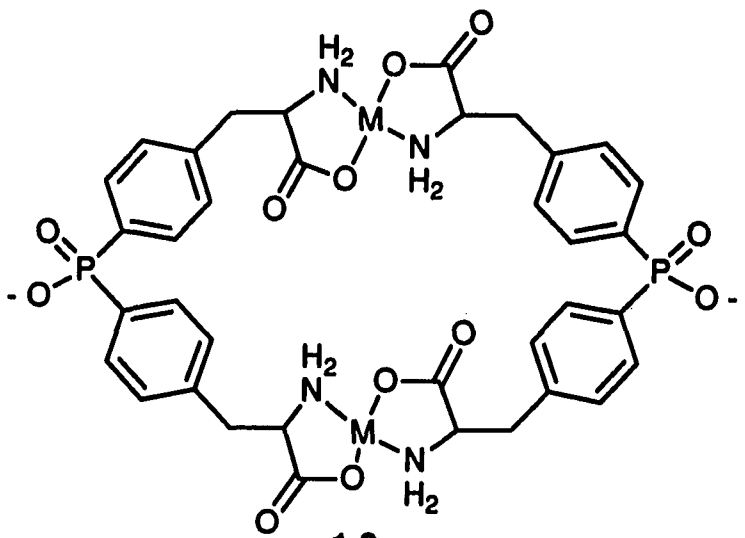
12

At pH = 10 in aqueous borate buffer, the complexation of ethylenediamine and Cu^{2+} leads to the closing of a hydrophobic pocket. The alkane chains in this complex form the lipophilic site, whose ability to hydrophobically bind aromatic hydrocarbons is enhanced by the ammonium ions on the chains. Addition of this complex **12** to a naphthalene solution leads to upfield shift of the naphthalene a-proton in the ^1H NMR spectrum. This is evidence for intracavity inclusion of the hydrocarbon in the complex **12**.

In the trianionic form of 4,4'-hydroxyphosphinylidine bis phenylalanine **11**, two amino groups and two acid groups function as metal binding sites. The complexation of two molecules of bisphenylalanylphosphinic acid and two metal ions generates a presumed macrocyclic complex **13.**

We initially investigated the Cu complex because of its stability. Compound **11** was dissolved in water with one equivalent of copper sulfate and the pH of the solution was adjusted to 7 by adding KOH or NH_4OH. The resulting complex was purified by size-exclusion chromatography. The complex was run through Sephadex G-10 (for molecules MW 50-700) and G-25 (for molecules MW 1000-5000). Only one blue band appeared and was

collected. Because the retention volumn of this column was not calibrated we can not confirm the cyclic dimer structure. In UV-Vis spectra, Cu^{2+} alone absorbs light λ_{max} = 754 nm in neutral aqueous solution. When the ratio of $[Cu^{2+}]$ to [11] =1, the mixture hasλ_{max} = 620 nm. With addition of excess amount of Cu^{2+}into the mixture,the free Cu^{2+} ion showed large absorption at longer wavelength.

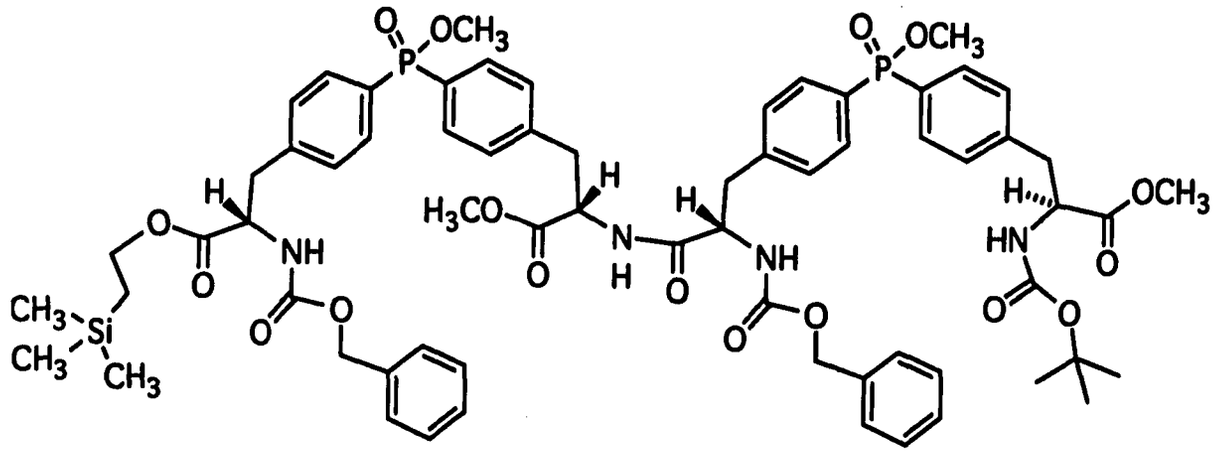


13

The square planar complex **13** represents a new class of self-assembled binding site. Cu^{2+} can be replaced by other transition metal ions to complex with bis amino acid **11** in various geometries. In this class of binding site, four benzene rings are folded to a hydrophobic binding cavity. Two phosphinic acid groups which provide the water solubility are remote from the hydrophobic cavity.

Bisphenylalanylphosphinic dipeptide

Connection of two molecules of bisphenylalanylphosphinate by an amide bond produces dipeptide **14**. The two open ends of the dipeptide can be closed by formation of a metal complex or by another peptide bond.

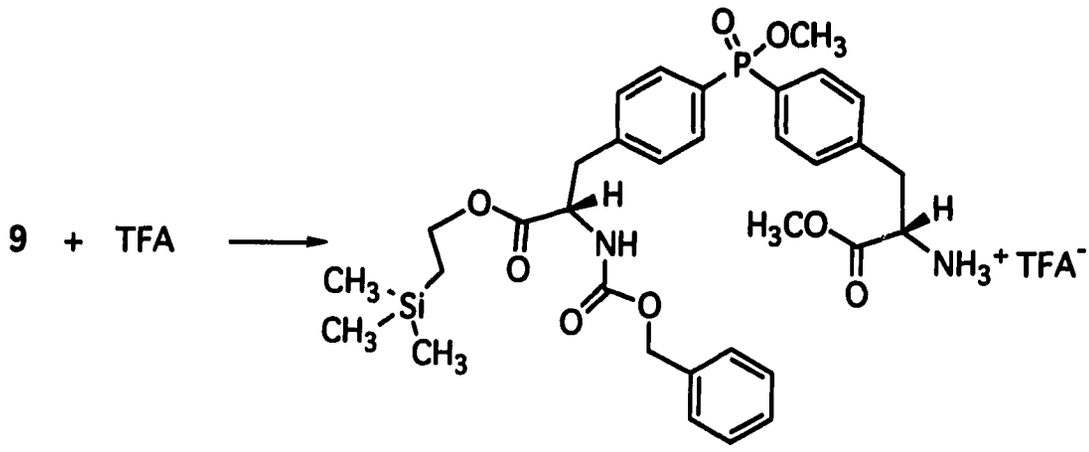


14

The complexation of the dipeptide and metal ion should be easier than that of bisphenylalanylphosphinic acids and metal ions which has been described above because less entropy change is required, and the resulting complex may be more rigid. DEprotection and metal binding would be an interesting subject for further research.

The synthesis of the dipeptide **14** from protected bisphenylalanylphosphinate involves deprotection, activation and coupling steps.

Deprotection The *t*-Boc protecting group was easily removed from unsymmetrical bisphenylalanylphosphinate **9** by the treatment with 5% trifluoroacetic acid (TFA) in CH_2Cl_2 (Scheme12).

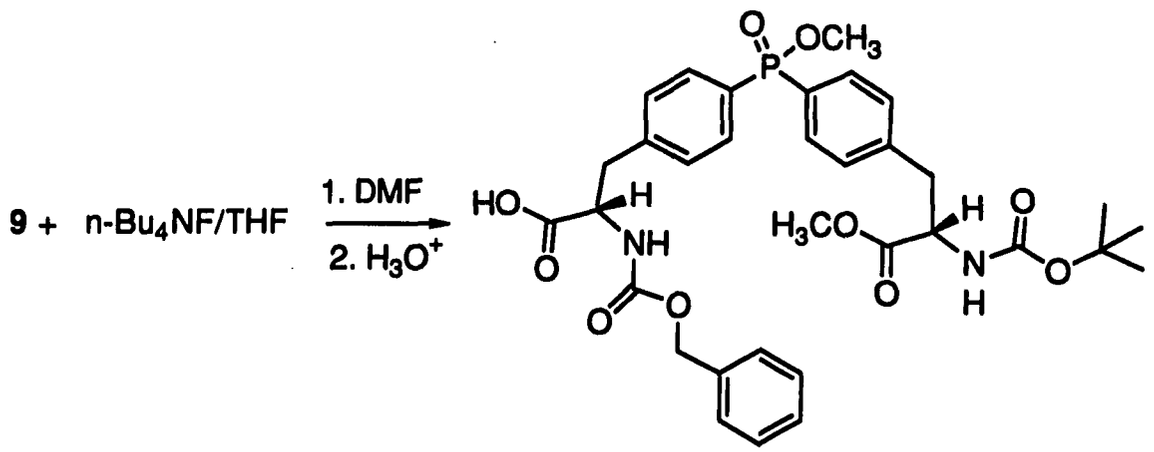


15

Scheme 12.

Excess TFA and solvent were removed under reduced pressure. The ammonium trifluoroacetate salt **15** was obtained as product in about 100% yield. In dilute TFA solution, the trimethylsilylethyl ester group remains unattacked, but in higher concentration of TFA (e.g. 15% TFA), this group can be cleaved.

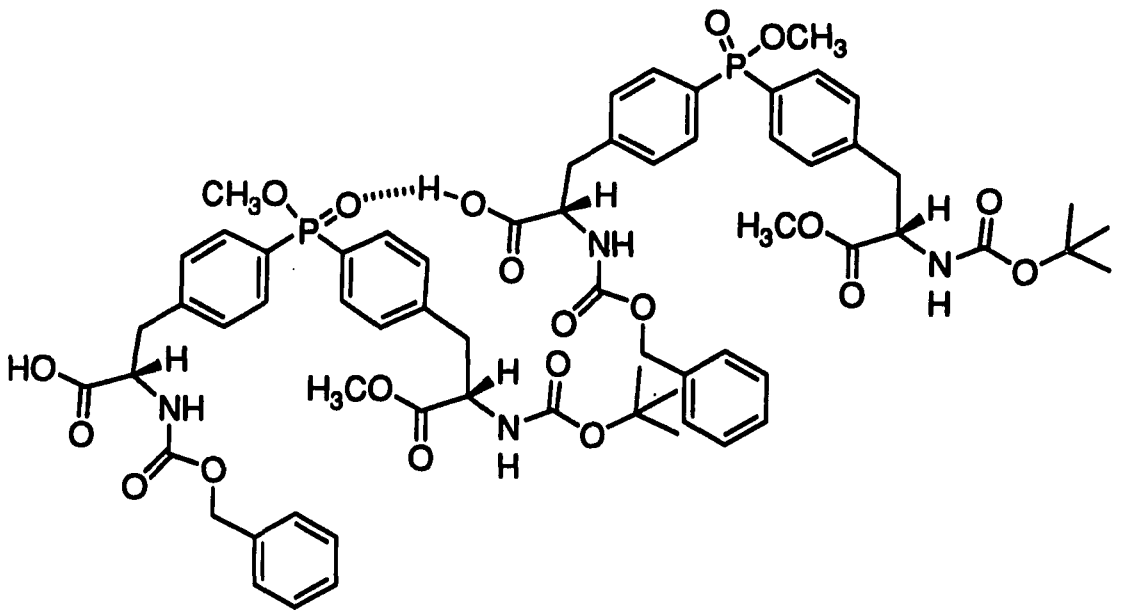
Removal of the trimethylsilylethyl ester group was accomplished by treatment with tetra-*n*-butylammonium fluoride/THF solution in DMF and followed by acidification (scheme 13). This reaction is usually performed in about 90% yield.



16

Scheme 13.

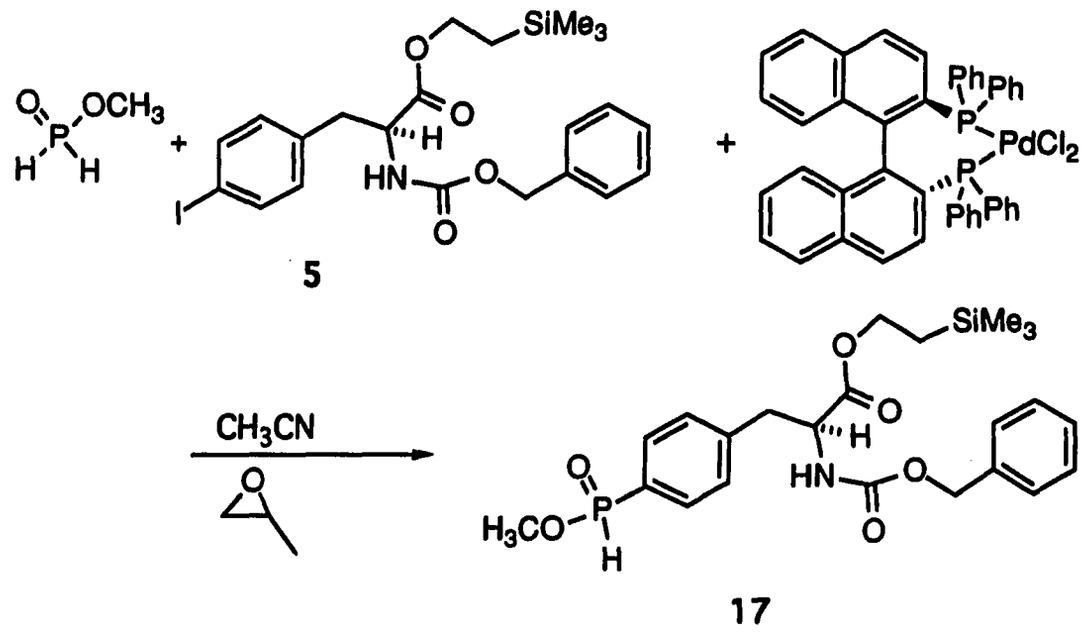
It is very interesting to see two diastereomers of the product **16** resolved in the ^{1}H NMR spectrum. Phosphorus is a chiral center in the unsymmetrical bisphenylalanylphosphinates. When the bis amino acid is in fully protected form, as in compound **9**, the two diastereomers are not resolved in ^{1}H NMR because of the closeness of their chemical shifts. Once the carboxylic acid protecting group is removed, the free acid group can hydrogen bond to the oxygen adjacent to the phosphorus (Scheme 14), behaving as a chiral shift reagent. In the ^{1}H NMR of acid **16**, the methoxyl groups adjacent to phosphorus show four signals that respond to two different methoxy doublets. The four signals are δ = 3.748, 3.731, 3.711, 3.678 with coupling constants J = 11.1 and 15.9 Hz. The two diastereomers of **16** show two peaks in ^{31}P NMR δ 35.760 and 35.577.



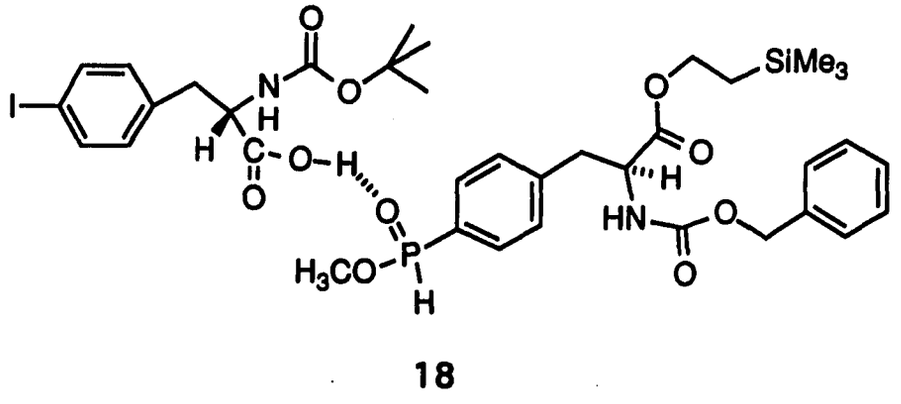
Scheme 14

We were curious whether a chiral catalyst would lead to enantioselectivity in the reaction of methyl phosphinate with an aryl iodide. We chose monophenylalanylphosphinate **17** formation and (R)-(+)-2,2'-bis(diphenylphosphino)-1,1'-binaphthyl-palladium(II) chloride as the chiral catalyst (Scheme 15).

Two diastereomers were measured by ^{31}P NMR using *t*-Boc-L-iodophenylalanine as a chiral shift reagent(Scheme 16). First, a mixed sample of N-Boc-iodo-L-phenylalanine and the monophenylalanylphosphinate**17** made from the achiral palladium catalyst was analyzed by ^{31}P NMR in C_6D_6 and CCl_4 mixture. Two ^{31}P signals appeared in a ratio about 1: 0.9 corresponding to the two diastereomers. That means with the achiral palladium catalyst, the arylphosphinate reaction does not have stereoselectivity.



Scheme 15.



Scheme 16.

A similar ^{31}P NMR experiment was carried out with the monophenylalanylphosphinate**17** which was made using the chiral palladium catalyst. The same ratio of ^{31}P NMR signals was observed, providing no

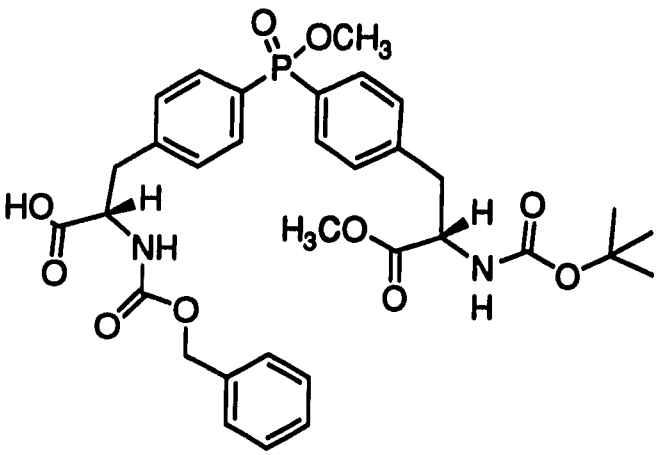
evidence for enantioselective reaction. Replacing the small methyl group with a larger ester group could be a direction for further investigation.

Activation and coupling Formation of an amide bond between two amino acids is an energy requiring process. Furthermore, peptide synthesis is usually performed at or below room temperature. Therefore, one of the groups that will produce the desired amide must be activated. A large number of activation methods have appeared in the literature. The basic requirements for activation methods are high yield, low racemization and no side-reactions. Usually the activated ester can be carried on to the peptide coupling step without isolation.

The first coupling reaction we tried was making hydroxybenzotriazolyl ester by treating diphenylalanylphosphinate free acid **16** with 1-hydroxybenzotriazole (HOBt) in the presence of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. The active ester reacted with bisphenylalanylphosphinate free amine **15** *in situ* to form dipeptide **14** (Scheme 17).

1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride, a water soluble carbodiimide, is a coupling reagent in the reaction. Unfortunately, this dipeptide **14** was formed in very low yield.

A similar reaction, dicyclohexylcarbodiimide (DCC) instead of the water soluble carbodiimide, was run on a model system. N-Cbz-iodo-phenylalanine **6** was treated with HOBt and DCC. The resulting active ester reacted with bisphenylalanylphosphinate diammonium TFA salt **15** without isolation. Peptide **19** was formed in 32% yield (Scheme 18).



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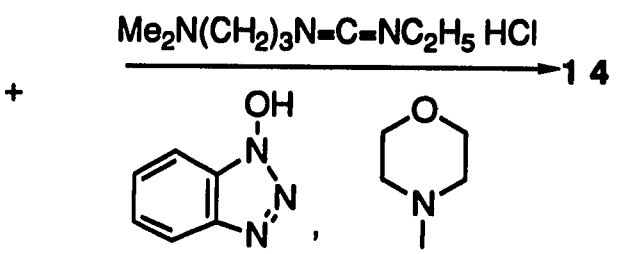

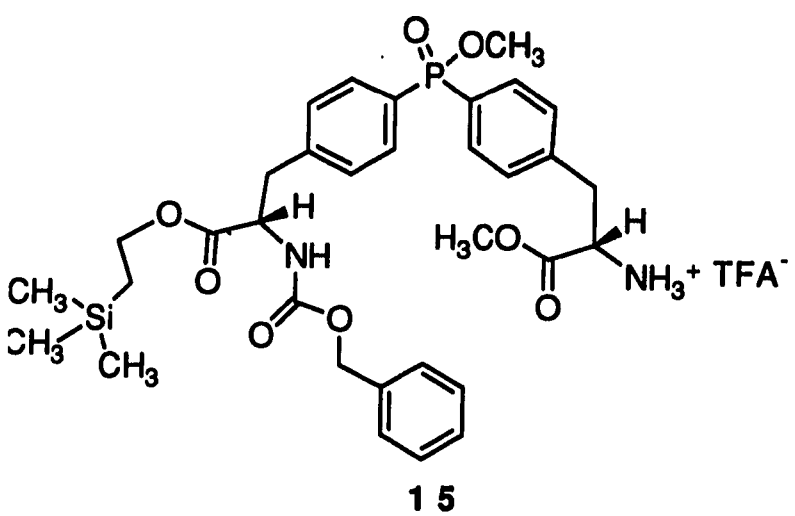

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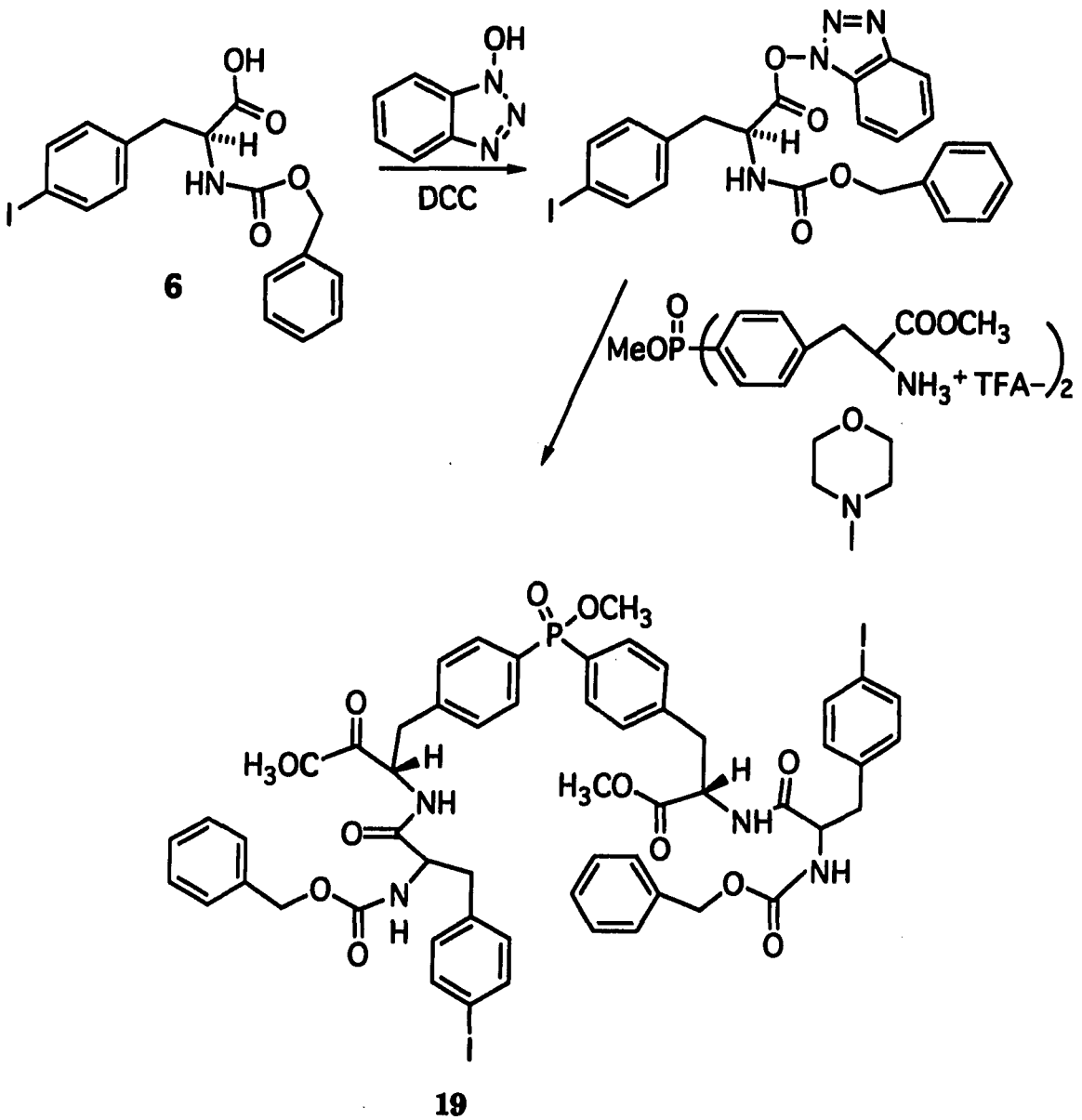
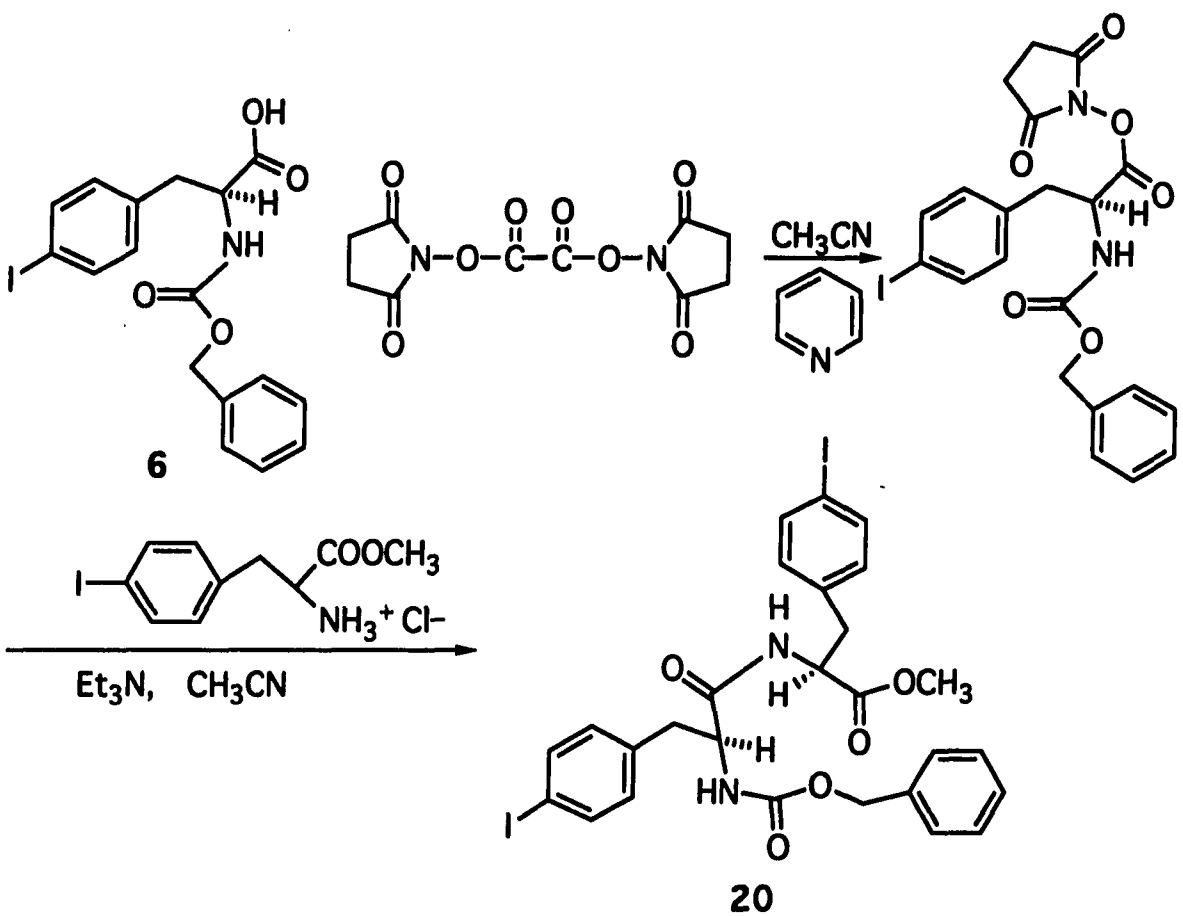
Scheme 17.



Scheme 18.

Oxalates have been demonstrated as convenient activating reagents in peptide synthesis without using coupling reagent such as DCC.[16] Disuccinimidyl oxalate was used in a model reaction. The crude dipeptide **20** was obtained in 78% yield. This process was performed by a nucleophilic

attack of carboxylate anion of N-Cbz-iodophenylalanine **6** on the carbonyl group of oxalate. The attack of the released alcohol on the intermediate formed the active ester which was converted in turn into peptide (Scheme 19).

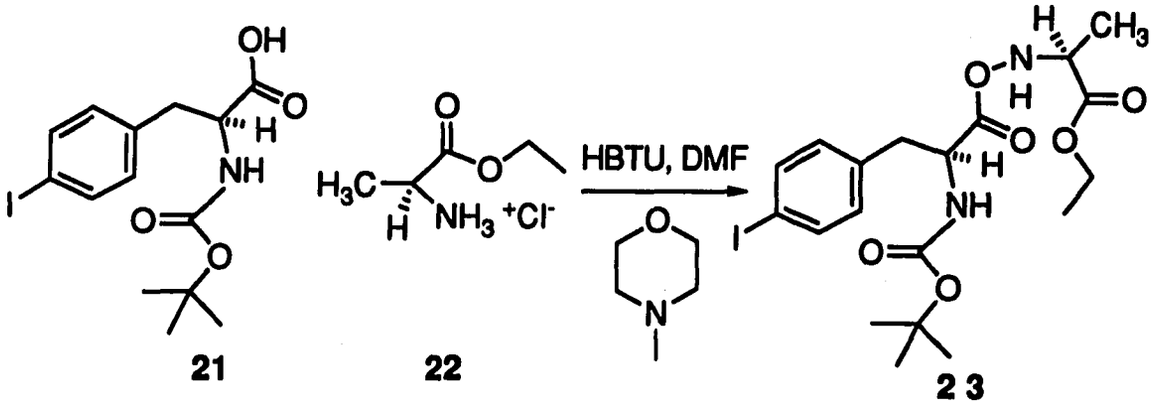


Scheme 19.

2-(1-Hydroxybenzotriazol-1-yl)-1,1,3,3,-tetramethyluronium hexafluorophosphate (HBTU) is an effective coupling reagent widely used in peptide synthesis.[17] It has following advantages comparing with other methods: <1> The amount of racemization of the synthetic peptides is very

low. <2> The coupling conditions are very simple: the coupling is achieved by mixing a solution of the N-protected amino acid and of the amino acid ester with HBTU in stoichiometric amounts in the presence of a tertiary amine base. <3> The reaction time is short and yields are high.

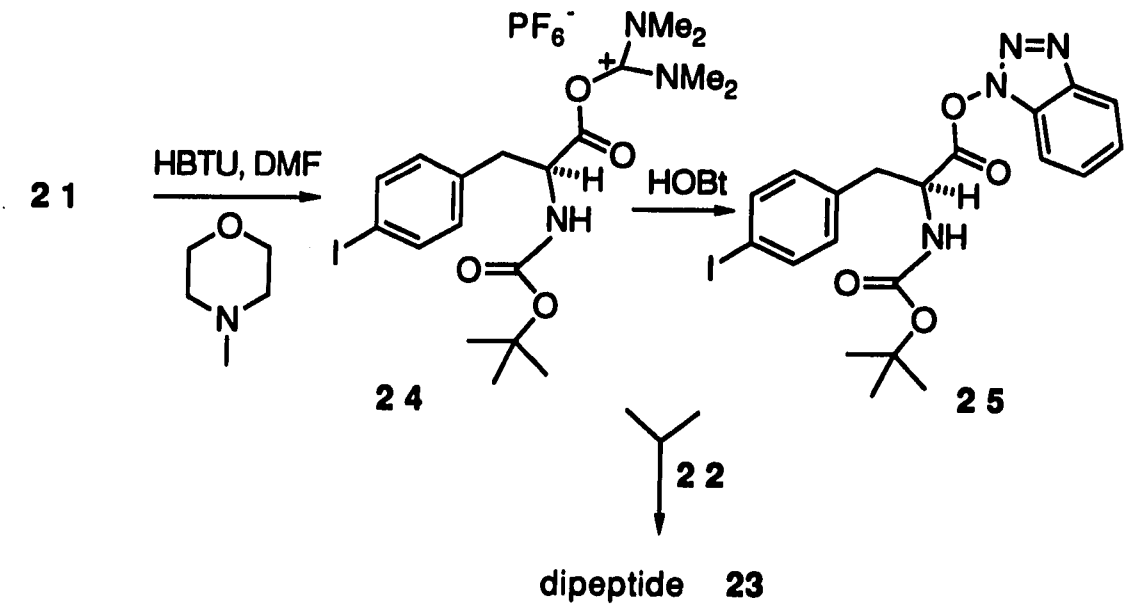
HBTU was used in a model reaction (Scheme 20). Crude dipeptide **23** was obtained in 86% yield.



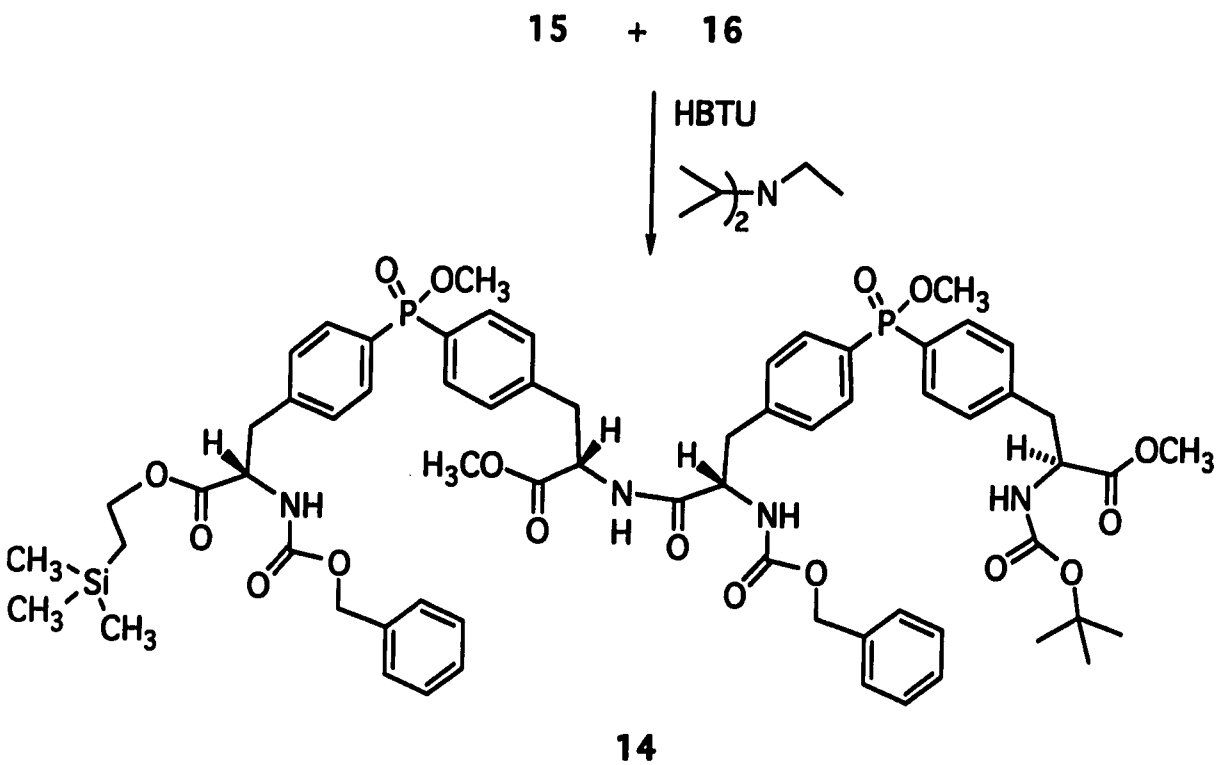
Scheme 20.

The peptide bond formation by the action of HBTU involves two intermediates, active ester of benzotriazole **25** and the tetramethyluronium salt **24**. Amino groups attack intermediates to form peptide bonds (Scheme 21).

Compared to the other coupling conditions we tried, the HBTU method gave the highest yield with a simple procedure. This method was applied to the synthesis of the designed dipeptide (Scheme 22). The desired dipeptide **14** was purified by flash chromatography and obtained in 76% yield.



Scheme 21.



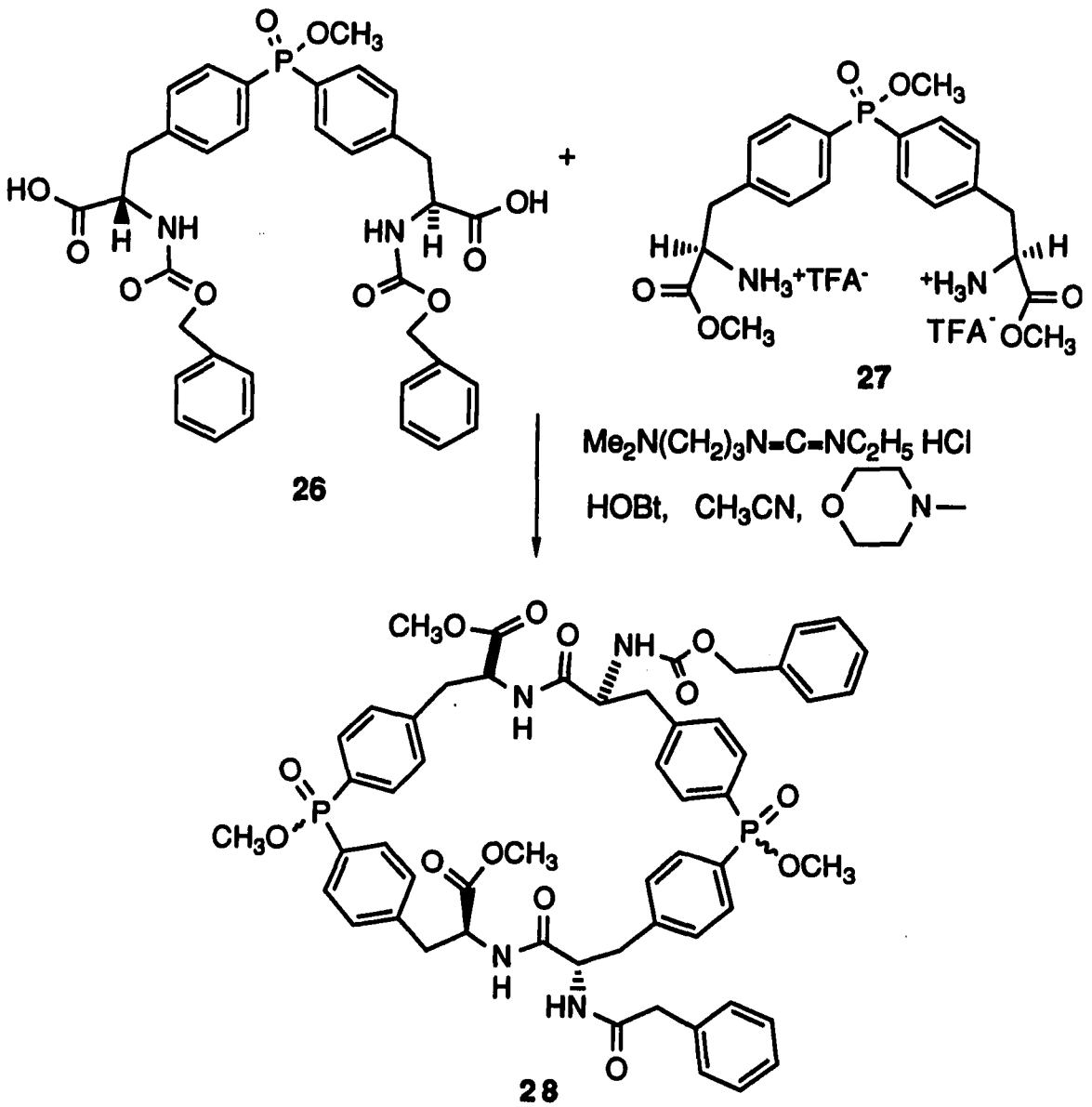
Scheme 22.

The macrocyclic peptide

Two molecules of bisphenylalanylphosphinate cyclized through two covalent bonds would produce a stable, preorganized macrocycle **28** or **32** which has a hydrophobic binding cavity and several potential water solubility providing groups. In principle two different approaches are possible for the synthesis of this macrocycle. For example, two peptide bonds can be formed in a single operation to generate a cyclic structure or two peptide bonds can be made separately. These two strategies have been investigated.

In the one step cyclization, two free carboxylic acid groups of one molecule of bisphenylalanylphosphinate couple with two free amino groups of another molecule of diphenylalanylphosphinate to form a cyclized structure **28** with two peptide bonds. The advantage of the one step synthesis would be its fewer steps. Symmetrical diphenylalanylphosphinate could be used in this method, which simplifies the sequence.

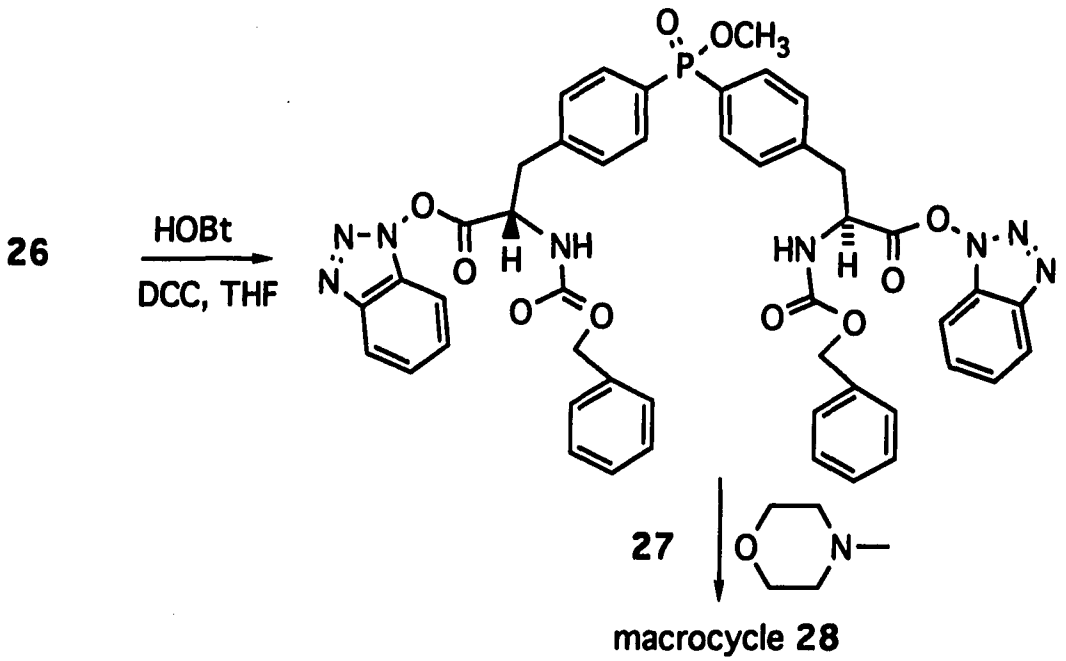
The deprotection of bisphenylalanylphosphinate **7** to **27** with TFA and **8** to **26** with n-Bu_4NF was carried out with the procedures mentioned previously. HOBt method was applied to this cyclization in the presence of a coupling reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride. Both free acid **26** and free amino **27** were mixed with HOBt and carbodiimide (Scheme 23). Unfortunately this one-pot reaction did not give the cyclized product.



Scheme 23.

Then we tried to make hydroxybenzotriazolyl diester with the aid of DCC. This active diester was then treated with the free amine **27** (Scheme 24). A mixture was formed in the reaction, But desired product **28** was not found. We

tried this reaction before finding HBTU procedure. If HBTU was used for this reaction, the cyclic product **28** might be generated.



Scheme 24.

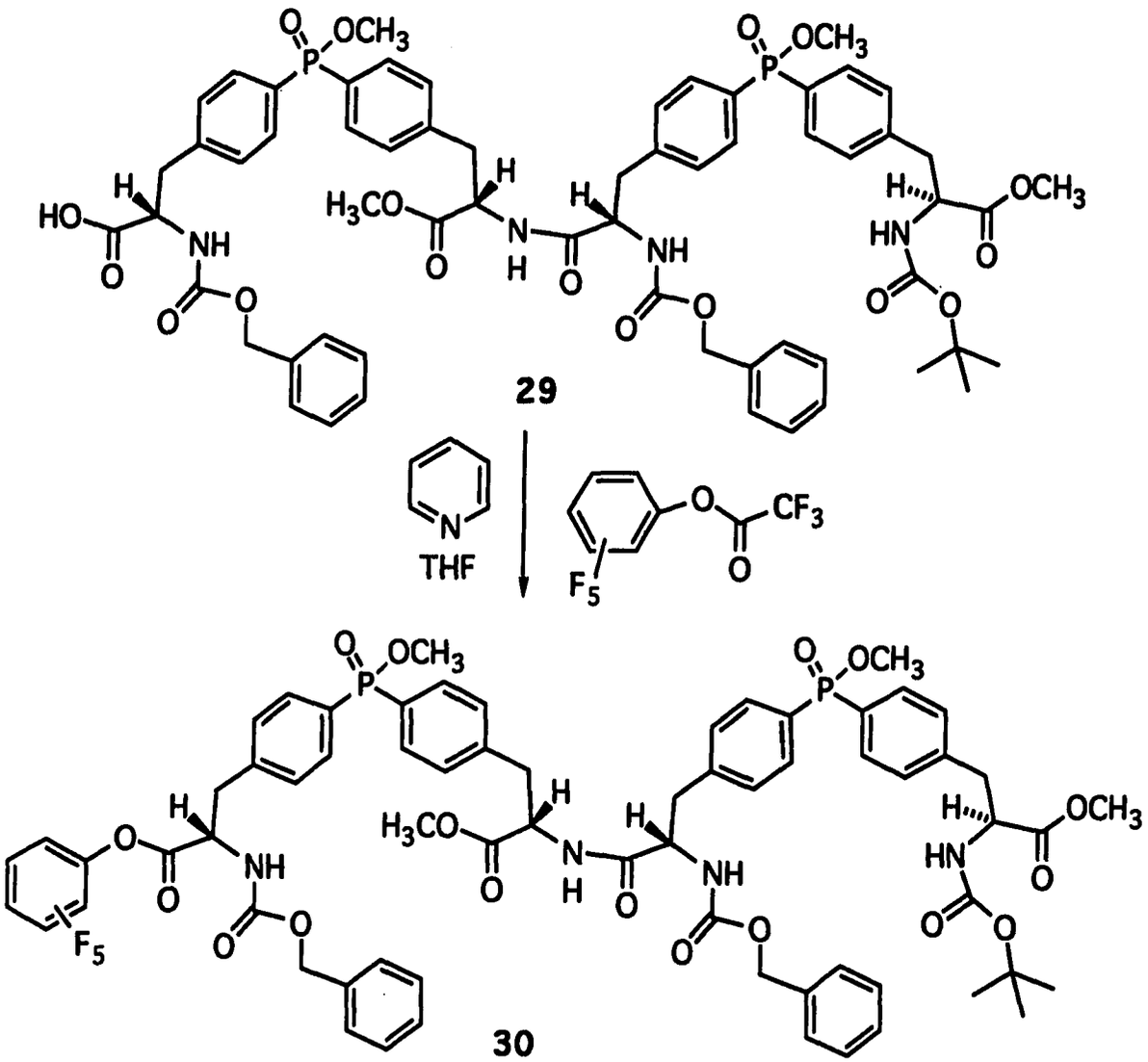
Macrocyclization is usually a low yield reaction because of the competitions of dimerization and polymerization. The higher concentration of the reactants, the lower yield of cyclized product. The one-step cyclization involved a bimolecular process which prefers high concentration of the two reactants. That is contradictory to the dilution principle which is widely used in the synthesis of macrocycles. Based on this concern, we changed our approach to the two step reaction.

In the two step process, bisphenylalanylphosphinate dipeptide **14** was made in the way that was described above. After removal of the

trimethylsilylethyl protecting group, the carboxylic acid of the dipeptide was activated in the form of a pentafluorophenyl ester for the cyclization. The use of pentafluorophenyl esters as preformed active esters for coupling reactions has become common, especially in the area of solid-phase peptide synthesis. Pentafluorophenyl ester has been compared with other activated esters and found it to be the best.[18] The standard preparation of these activated esters is DCC mediated coupling of the N-protected amino acid and pentafluorophenol. Green and Berman reported a more convenient alternate route to these esters by using pentafluorophenyl trifluoroacetate as activating reagent.[18] This new procedure offers high yields, short reaction time and great convenience. Pentafluorophenyl trifluoroacetate has been chosen as activating reagent in our synthesis. The activated ester **30** was prepared by treating the dipeptide free acid **29** with pentafluorophenyl trifluoroacetate and pyridine in THF. The reaction went completion in 10-15 min. The activated ester was purified by flash chromatography and obtained in 76% yield (Scheme 25).

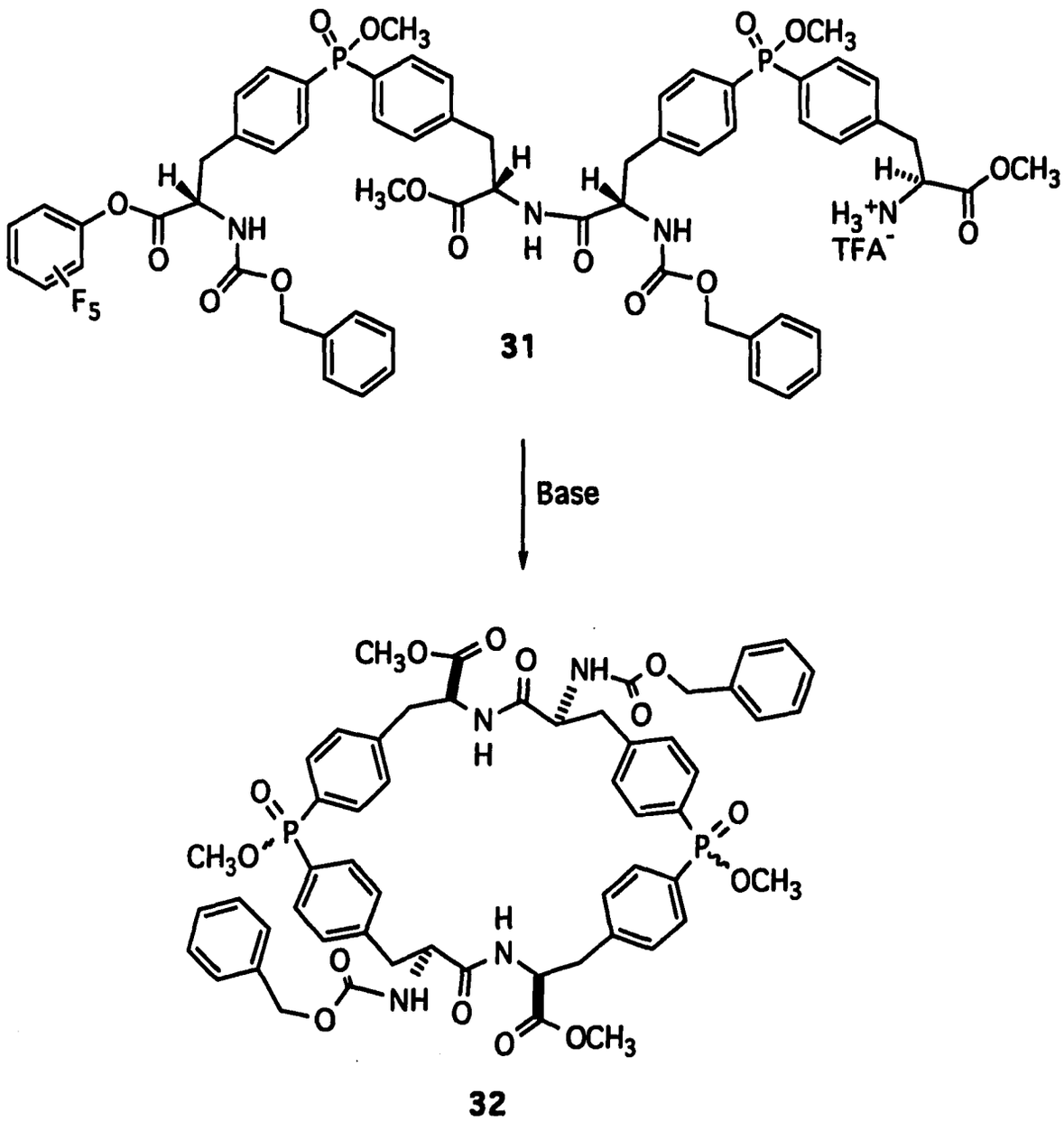
We found that pentafluorophenyl trifluoroacetate can easily be hydrolysed with trace concentrations of water even at low temperature. The reaction of making amino acid pentafluorophenyl ester is extremely sensitive to water. All the reagents and solvent must be freshly distilled. Especially, pyridine has to be distilled from fresh CaH_2. In order to avoid product hydrolysis the product was purified by flash chromatography, but not by the aqueous wash procedure described by Green and Berman. The purified product was carried on to the next reaction immediately. The next step of removing *t*-Boc protecting group was carried out in dilute TFA solution. The resulting

ammonium TFA salt **31** was dried under vacuum. This product was ready for the cyclization.



Scheme 25.

The peptide cyclization was carried out in dilute solution in the presence of a tertiary amine as base.



Scheme 26.

The precursor **31** of the cyclization should be very pure because any impurity could cause side reactions. N-methylmorpholine and diisopropylethyl

amine have been used as bases. The hindered more basic diisopropylethylamine worked better than N-methylmorpholine. The cyclization was originally carried out in THF or CH_2Cl_2, and the product **32** yield was around 20-30%. Since amide bond formation usually works the best in DMF, we switched to use DMF. The reaction yield increased to 38%.

The method of adding the precursor to the basic solution is very critical in this cyclization. When the precursor molecule is in a diluted solution, few molecules are nearby and the only reaction pathway is the intramolecular attack to form the cyclized product. If the molecule has a chance to contact other molecules in the presence of base, the dimerization or polymerization will definitely be the dominant processes. When we first tried the cyclization, the precursor was dissolved in a large amount of solvent. This very dilute solution (0.06-0.09 mM) was added to a dilute base in a long period of time (>8 h). After the addition finished, some unreacted dipeptide was found which further underwent dimerization or polymerization. This method failed to give the cyclized product in good yield. When the precursor was in a very dilute environment, it did not have a chance to contact either other molecules of the precursor or the base. No reaction could occur in this situation except hydrolysis. The accumulated the reaction precursors underwent undesirable hydrolysis, dimerization or polymerization. From these results, we realized that even though dilution is necessary in this type of reaction, the slow addition of the precursor could be more important. The improved method was using syringe pump to add precursor **31** slowly into a basic DMF solution. The addition rate can be controlled precisely. Compound **31** was dissolved in DMF (~19 mM) and added to the solvent containing base (~0.8 mM) by syringe

pump very slowly (0.0097 ml/min). The outlet tubing from the syringe pump was inserted into the basic solution so that the reactant solution did not accumulate at the end of the tubing. The best reaction yield (38%) was obtained in this method. Raising the reaction temperature did not improve the rate or yield of the cyclization, but could cause racemization.

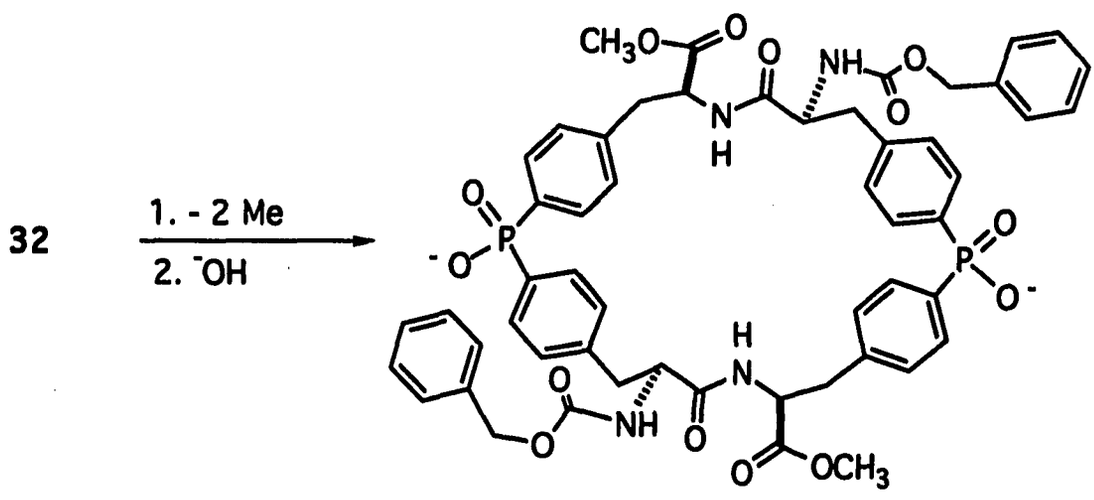
The macrocyclic peptide **32** was purified by flash chromatography and characterized by ^{1}H NMR, ^{31}P NMR, IR and FAB-mass spectroscopic methods.

Deprotection of the Macrocyclic Peptide

The macrocyclic peptide **32** bears several water solubility providing groups. In the synthetic process described previously, these groups are in the protected forms. The last step, removal of protecting groups, will provide the potential water soluble hydrophobic binding sites.

Cleavage of methyl arylphosphinates to arylphosphinic acids was chosen as the first approach. In neutral aqueous solution, the macrocycle is in anionic form **33** (Scheme 27). The anionic groups are positioned away from the aromatic walls so that they do not interfere the hydrophobicity of the binding sites.

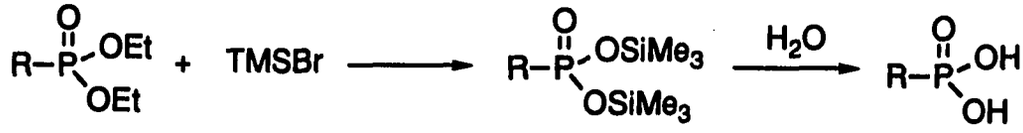
The deprotection should be selective to only remove the methyl ester groups without touching other functionalities in the molecule. Bromotrimethylsilane (TMSBr) has been reported to be a highly selective reagent for P-O silyldealkylation of phosphonate alkyl esters by McKenna and Schmidhauser.[19]



33

Scheme 27.

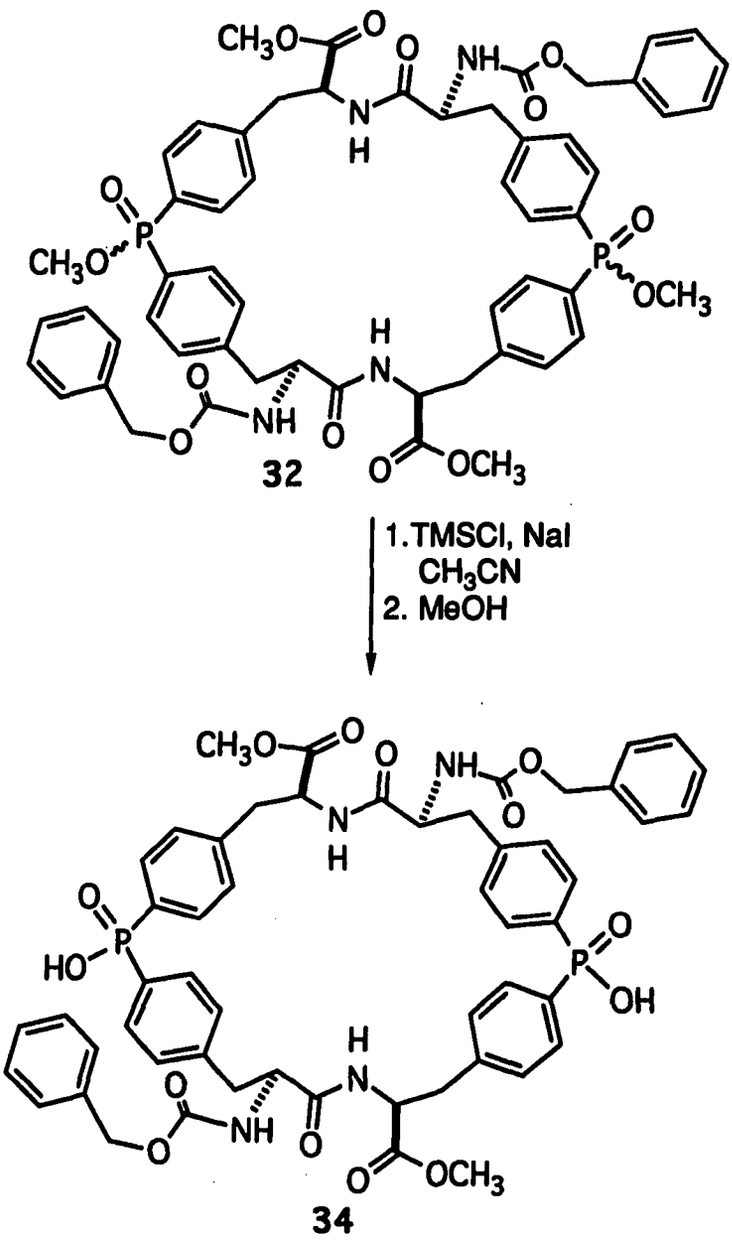
They showed that TMSBr is compatible with carboxylate groups in the phosphonate esters. The products, phosphonate trimethylsilyl esters, are readily converted to corresponding phosphonic acids by hydrolysis with neutral H_2O (Scheme 28).



Scheme 28.

TMSBr has been used to cleave methyl ester groups in our macrocyclic system. Methyl ester of phosphinate can be replaced by trimethylsilyl group in CH_3CN at room temperature, but benzyloxycarbonyl, an amino protecting group, was also partially cleaved at the same time as determined by ninhydrin detection of free amine. Two methods were tried to slow down the cleavage of

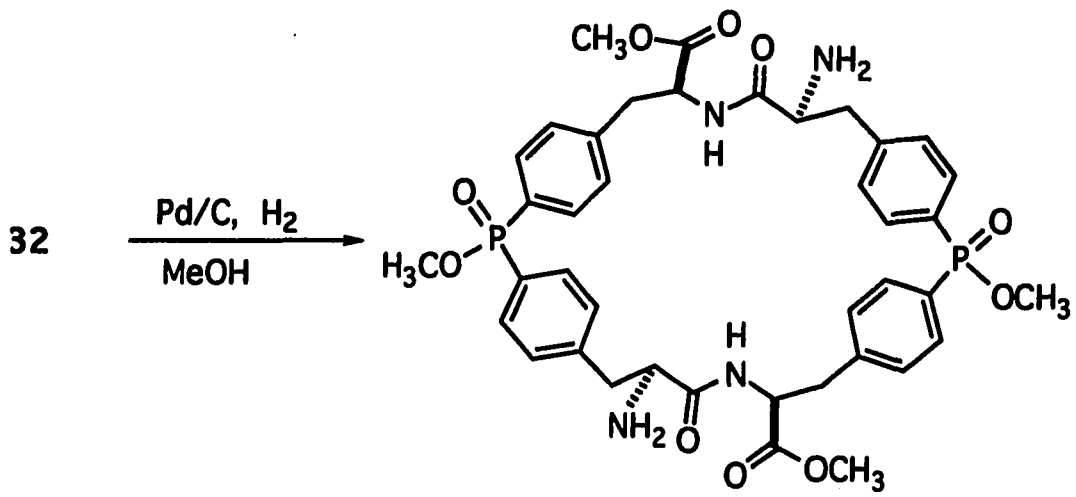
the Cbz group . The first one is adding pyridine to the reaction mixture to scavenge HBr, which is the hydrolyzed product of TMSBr. However, pyridine also inhibited the deprotection reaction. The demethylation did not occur when there was more than 0.25 equivalent of pyridine in the reaction mixture. A smaller amount of pyridine could not prevent the Cbz groups from cleavage effectively. The second method is to carry the reaction at low temperature. Cbz groups seem stable at 0-5°C to the TMSBr treatment. But the demethylation is also slowed down at low temperature. The reaction did not go to completion at 0-5°C. The mass spectrum of the product showed unreacted, partially deprotected and completely deprotected macrocycles. Iodotrimethylsilane (TMSI) has been demonstrated as a more reactive dealkylation reagent for phosphonate esters than TMSBr.[20-22] In the dealkylation reactions, TMSI can be generated *in situ* by chlorotrimethylsilane (TMSCl) and sodium iodide. The deprotection of **32** with TMSI was curried out at 50°C in CH_3CN solution. The product trimethylsilyl ester was treated with MeOH to give the corresponding phosphinic acid **34**. This acid was purified by reverse phase flash chromatography and HPLC with a C-18 column. The purified product was characterized by ^1H NMR, ^{31}P NMR and FAB-mass. The macrocyclic phosphinate in its ester form **32** shows three ^{31}P NMR signals which we believe are caused by the chirality of the phosphorus atoms. When the methyl esters are cleaved, the phosphorus atoms are no longer chiral centers: the macrocyclic phosphinic acid **34** shows only one signal in the ^{31}P NMR.



Scheme 29.

The macrocyclic peptide **32** can also undergo hydrogenolysis to remove the Cbz amino protecting groups. In a preliminary study, we found that the hydrogenolysis was a slow process at room temperature even in the presence 0.5

eq palladium in the reaction mixture. The deprotected macrocycle **35** with free amino groups is adsorbed on carbon and can be only rinsed out with acidic aqueous solution. This product was not isolated.



35

Scheme 30.

This deprotected macrocycle is also a potential hydrophobic binding site with H_2O solubility providing groups on the two peptide linkers. The advantage of macrocycle **35** compared with **33** is that two large Cbz groups are removed which could block the cavity and cause aggregation.

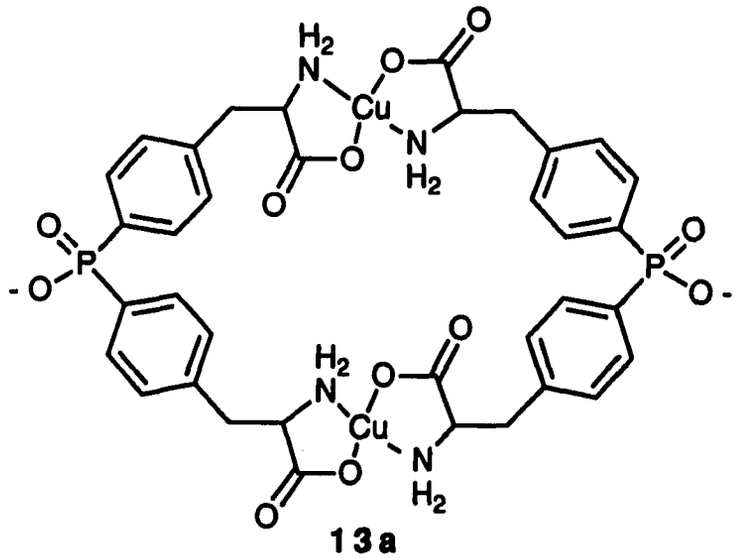
Preliminary Binding Study

Preliminary binding studies using the potential binding sites described above, have been carried out by using UV, fluorescence and NMR techniques.

Metal complex

In the complexation study of 4,4'-hydoxyphosphinylidine bis phenylalanine (PBP), Cu^{+2} was chosen as metal ion because of the square planar geometry of copper complexes. NMR technique is not suitable for this study because paramagnetic Cu^{+2} causes NMR signal broadening. Fluorescence and UV titrations have been used to measure the binding of this self-assembled macrocyclic complex with substrates.

The complex **13a** formed as described above was studied in N-2-hydroxyethylpiperazine-N'-2-ethane sulfonic acid (HEPES) (pH 7, 100 mM) buffer solution. From CPK model examination, this complex **13a** possesses an anthracene sized cavity, the walla of which are formed by the copper chelates.



13a

Preliminary studies, by UV titration, with the ligands pyrazine and 1,4-diazabicyclo[2.2.2]octane (DABCO) and the hydrophobic methyl orange and ANS gave no evidence of binding.

Fluorescence titration has been performed with this complex by using ANS as substrate. In hydrophobic organic solvents or complexed in nonpolar host cavities in water, ANS exhibits notable increases in fluorescence and blue wavelength shift. If ANS binds in the cavity of the (PBP-Cu)2 complex, both macrocycle **13** formation and its binding with ANS can be determined by increasing fluorescence intensity. Only very slight fluorescence intensity change has been observed during the titration. The results are shown in Figure1. If binding takes place, the enhanced fluorescence in the less polar environment may be compensated by quenching by Cu^{2+}.

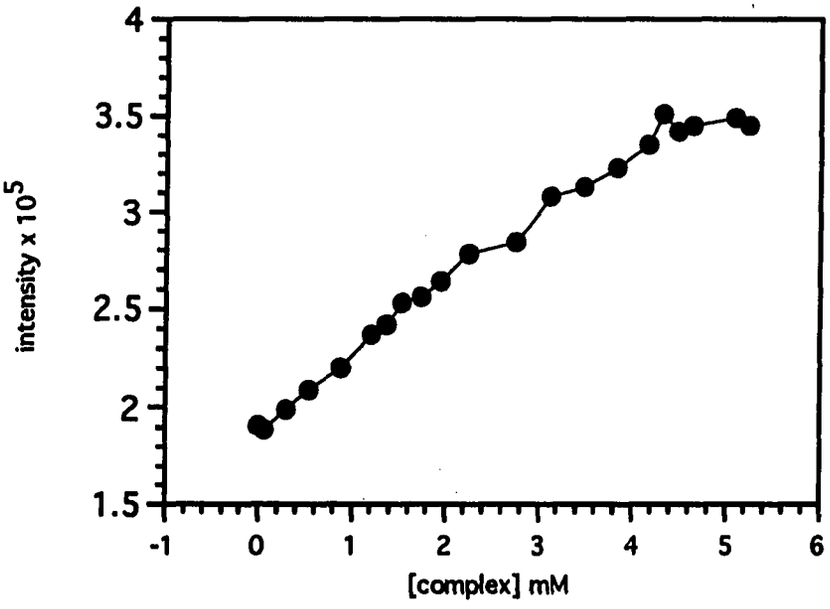


Figure 1. Fluorescence intensity change in the titration of ANS (0.01mM) with complex **13a** at pH 7, λ(exitation)=375 nm, λ(emission)=500 nm

The fluorescence data hint at complexation, but are not of sufficient quality to determine binding. Study of metal PBP complexes was continued by Jinho Lee. Using U-tube transport experiments not dependent on spectroscopic changes, he demonstrated binding of pyrene by the Co^{2+} and Ni^{2+} PBP complexes.[23]

Macrocyclic peptide

The macrocyclic phosphinic acid **33** may be a hydrophobic binding site in neutral aqueous solution. The aggregation of **33** was examined by dilution of **33** aqueous solution at pH 7 from 13 mM to 0.2 mM. No ^{1}H NMR chemical shift change has been observed in this concentration range. Below 0.2 mM, ^{1}H NMR signals of **33** could not be detected. We assume the CAC of **33** is above 13mM.

NMR titration has been applied to study the binding of **33**. Pyridine and pyrazine have been chosen as substrates. In aqueous solution of **33** at pH 7 the ^{31}P NMR signal of **33** showed upfield shift with increasing the concentration of pyridine or pyrazine (Figure 2 and Figure 3). But the titration curve did not show saturation. When the same titration was applied to a model system, potassium diphenylphosphinate, an upfield shift of ^{31}P NMR signal of methyl diphenylphosphinic acid was also found. The observed ^{31}P NMR signal upfield shift is due to a solvent effect, and does not provide evidence of binding.

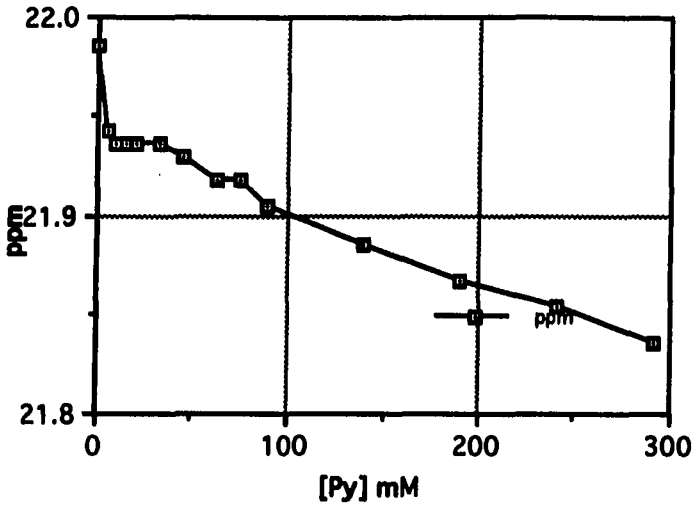


Figure 2. ^{31}P NMR chemical shift change with increasing the concentration of pyridine in the macrocycle **33**/D_2O solution, [**33**]=8.6 mM, pH=7

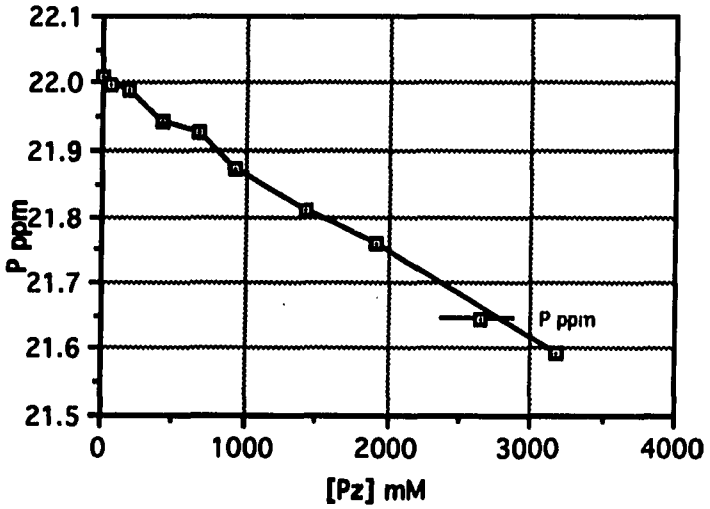


Figure 3. ^{31}P NMR chemical shift change with increasing the concentration of pyrazine, pH=7, in D_2O, [**33**]=12 mM

Anthracene, naphthalene and 2,7-dihydroxynaphthalene were also tried as substrates in the ^1H NMR titration. Anthracene did not show ^1H NMR signals because of its low solubility in D_2O. Naphthalene in D_2O solution showed its most down field aromatic signal at δ 7.97. When 10.1 mM macrocycle **33** in D_2O was stirred with naphthalene, no aromatic signal was observed downfield of δ 7.70, implying that the naphthalene signal shifted from δ 7.97 to δ< 7 70. The range of naphthalene chemical shift change could not be determined because of the overlap of the host and the guest signals. But this is the first hint of binding by **33**. Since 2,7-dihydroxynaphthalene has a higher solubility in water than naphthalene, we choose this compound as a relative more soluble substrate. Unfortunely, no ^1H NMR chemical shift change of 2,7-dihydroxynaphthalene in the presence of 27.8 mM macrocycle **33** was not observed, suggesting that binding did not occur. This is in contrast to the behavior of receptors prepared by Koga and Diederich with the same connectivity as ours, but could possibly be due to the geometry of the OH groups.

One of the reasons why this macrocyclic peptide **33** did not show binding property as it was expected may be the purity of the macrocycle. The impurities in the macrocycle could interfere with the binding. Recently we have found a purification procedure for **33** by HPLC . The purified macrocycle **33** is ready for the further binding study.

EXPERIMENTAL SECTION

General Procedure

See General procedure in PAPER I. HBTU was used as received from Applied Biosystems.

4-Iodo- L-phenylalanine

L-phenylalanine (10.00 g, 60.5 mmol) was dissolved in 70 ml HOAc and 7.5 ml sulfuric acid. This solution was poured into a flask containing iodine (5.84 g, 23.0 mmol) and sodium iodate (2.51g, 12.7 mmol). The mixture was heated to reflux until TLC (16:3:2.5 MEK:HOAc:H_2O/ninhydrin, product R_f=0.54, Phe R_f=0.46) showed the reaction to be complete (1-2h). The TLC sample was prepared by diluting one drop of reaction mixture with 1 ml water. HOAc was removed by rotary evaporation in a 35°C water bath to leave about 32 g of residue. The dark oil, dissolved in 60 ml water, was extracted with 5x10 ml CH_2Cl_2 to remove residual iodine. The aqueous solution was decolorized by boiling with 0.5 g norit, and filtered through celite. The filtrate was neutralized with conc. aqueous NH_3 to pH 7. After cooling in an ice bath, the white solid was collected by vacuum filtration, and washed with cold EtOH. This damp solid (about 70-80 g) was dissolved in 100 ml H_2O and 40 ml HOAc and precipitated by adding aqueous conc.NH_3 to pH 7. Purification was achieved by crystallization twice from acetic acid (30 ml and 50 ml) to yield 4-iodo-L-phenylalanine (8.8 g, 50%), identical to commercial iodophenylalanine by comparing ^1H NMR spectra and TLC, but having a higher mp 253-254 °C [Literature: 225-226°C (Serva)]. Purity was checked by

HPLC and optical purity was tested by chiral TLC with Chiralplate(Macherey-Nagel).[8] ^{1}H NMR (300 MHz, D_2O/DCl) δ 7.82(d, J=8.1Hz, 2H), 7.15(d, J=8.1Hz, 2H), 4.43(t, J=6.2Hz, 1H), 3.36(dd, J=5.8, 14.7Hz, 1H), 3.25(dd, J=7.5, 14.4Hz, 1H); ^{13}C NMR(75.429 MHz, D_2O+DCl) δ 175.34, 159.02, 136.84, 132.19, 130.15, 52.47, 19.34; IR (KBr, cm^{-1}) 2918, 1718, 1701, 1585, 1522, 1487, 1396.

N-benzyloxycarbonyl-4-iodo-L-phenylalanine trimethylsilylethyl ester 3

N-benzyloxycarbonyl-4-iodo-L-phenylalanine **6** (2.45 g, 5.78 mmol) was dissolved in 20 ml CH_3CN and 1.0 ml pyridine. 2-trimethylsilylethanol (0.82 g, 6.94 mmol) was added. The clear solution was stirred on an ice bath for 20 min. Dicyclohexylcarbodiimide (1.33 g, 6.36 mmol) was added to the solution. The mixture was stirred on an ice bath for 1 hour, then stored in a refrigerator overnight. Oxalic acid (0.15 ml of a 5M solution in DMF) was added to the mixture containing white solid and stirred for 1 hour to consume any remaining DCC. The precipitate was removed by filtration and washed with EtOAc. The organic filtrate was washed with 0.5N HCl and $NaHCO_3$ (satd.). The EtOAc layer was dried with $MgSO_4$ and filtered. Solvent was removed under reduced pressure. Product was purified by flash chromatography (1:9 EtOAc:Hexanes). A white solid was obtained in 88% yield mp 45-46.5°C (2.68 g). ^{1}H NMR (300 MHz,$CDCl_3$) δ 7.57(d, J=8.4Hz, 2H), 7.40-7.28(m, 5H), 6.85(d, J=8.4Hz, 2H), 5.28(d, J=8.4Hz, 1H), 5.11(d, J=12.3Hz, 1H), 5.06(d, J=12.3Hz, 1H), 4.59(q, J=5.7, 13.8Hz, 2H), 3.09(q, J=6.0, 14.1Hz, 1H), 2.99(q, J=6.3, 13.8Hz, 1H), 0.95(d, J=6.6Hz, 1H), 0.92(d, J=6.6Hz, 1H), 0.036(s, 9H);

^{13}C NMR (75 MHz, $CDCl_3$) δ 171.30, 155.51, 139.68, 137.56(2C), 136.19, 135.55 131.34(2C), 128.52(2C), 128.21, 128.08(2C), 92.55, 66.95, 64.06, 54.69, 37.81, 17.34; IR (NaCl, cm^{-1}) 3730, 3584, 3354, 2953, 1726, 1514, 1250, 1059, 839, 696.

N-*t*-Boc-4-iodo-L-phenylalanine methyl ester 5

Thionyl chloride (10.2 g, 0.086 mol) was added dropwise to 10 ml of methanol that was stirring on an ice bath. 4-Iodo-L-phenylalanine (5.0 g, 0.017 mol) was added to this methanol solution. This yellow solution was refluxed for 2h. TLC(16:3:2.5 MEK: Et_3N:H_2O, ninhydrin, product R_f=0.67, S.M. R_f =0) showed the reaction to be complete. The reaction mixture was rotary evaporated to a white solid, which was redissolved in about 10 ml of MeOH and crystallized by adding about 50 ml of ether. 4-Iodo-L-phenylalanine methyl ester hydrochloride **2** was obtained (5.40g, 92%). mp 199.5-200.5°C; ^1H NMR (300 MHz, CD_3OD) δ 7.73(d, J=6.0Hz, 2H), 7.05(d, J=6.0Hz, 2H), 4.31(t, J=6.9Hz, 1H), 3.81(s, 3H), 3.22(dd, J=15.0, 6.0Hz, 1H), 3.12(dd, J=15.0, 7.5Hz, 1H).

4-Iodo-L-phenylalanine methyl ester hydrochloride **2** (5.40 g, 0.0158 mol) in 30 ml CH_2Cl_2 was treated with N-methylmorpholine (4.8 g, 47.8 mmol) and di-t-butyldicarbonate (4.49 g, 0.0206 mol). The reaction mixture was stirred at RT under N_2 for 5h and followed by TLC (4:1 Hexanes:EtOAc, product R_f=0.30). During stirring, a white precipitate was formed. The reaction was worked up by stirring with H_2O. The precipitate dissolved in water. CH_2Cl_2 was removed under reduced pressure. The resulting yellow oil was dissolved in EtOAc and washed twice with satd. $NaHCO_3$, citric acid twice, H_2O and

satd.NaCl, then dried over Na_2SO_4. After filtration and removal of solvent by rotary evaporation, the white solid was left. Crude product was dissolved in about 10 ml of CH_2Cl_2 and crystallized from 50 ml of hexanes. White crystal was obtained as product (5.1g, 77%). mp 74-76°C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.61(d, J=8.4Hz, 2H), 6.88(d, J=8.4Hz, 2H), 5.04-4.95(m, 1H), 4.61-4.52(m, 1H), 3.72(s, 3H), 3.08(q, J=5.7, 13.8Hz, 1H), 2.98(q, J=5.7,14.1Hz, 1H), 1.42(s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 172.06, 155.01, 137.61(2C), 136.09, 135.74, 131.34(2C), 92.55, 54.21, 52.36, 37.93, 28.31; IR (NaCl, cm^{-1}) 3358, 2978, 1744, 1715, 1485, 1366, 1215, 1165, 1059, 1009.

N-benzyloxycarbonyl-4-iodo-L-phenylalanine 6

4-Iodo-L-phenylalamine (20.0 g, 68.7 mmol) and NaOH (4.25g, 106 mmol) were dissolved in 100 ml H_2O. The resulting mixture was stirred for 15 min and filtered to remove impurity. Filtrate was concentrated to about 50 ml. Benzyl chloroformate (13.48 g, 79.0 mmol) and 50 ml NaOH solution (4.75g, 118.8mmol) were added in approximately 10 portions alternately over a 30 min period. The solution turned cloudy. The reaction mixture was stirred at RT for one hour until completion and checked by TLC (16:3:2.5 MEK:AcOH:H_2O, product R_f=0.92, S.M. R_f=0.73). This solution was extracted with ether 3 x 30 ml. Aqueous solution was acidified with conc. HCl to pH 2. The product precipitated as a white solid, which was filtered and washed with water 3 times and hexanes 2 times, and then dried in vacuo. N-benzyloxycarbonyl-4-iodo-L-phenylalanine was recrystallized from 20 ml of CH_3CN and 100 ml of CH_2Cl_2 to yield 26.43 g (91%). mp 150-152°C; ^1H NMR (300MHz, CD_3CN) δ 7.63(d, J=8.1Hz, 2H), 7.31(m, 5H), 7.01(d, J=7.5Hz, 2H), 5.89(m, 1H), 5.02(s,

1H), 5.00(s, 1H), 4.39(m, 1H), 3.13(dd, J=13.8, 4.5Hz, 1H), 2.87(dd, J=13.8, 9.0Hz, 1H).

Compound 7

The preparation is described as compound **23c** of PAPER I.

4,4-(methoxyphosphinylidene)bis[N-[(phenylmethoxy) carbonyl]]-L-Phenylalanine di-2,2-trimethylsilylethyl ester 8

Procedure B (described in PAPER I) is followed starting with phosphinic acid (52 mg, 0.897 mmol), (S)-N-Cbz-*p*-iodophenylalanine 2,2-trimethylsilylethyl ester **3** (653 mg, 1.26 mmol), triphenylphosphine (26.5 mg, 0.101mmol), palladium acetate (5.7 mg, 0.025 mmol) and N-methylmorpholine (130 mg, 1.30 mmol). Flash chromatography (7:3 ethyl acetate:hexanes) gave 225 mg product (41%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ 7.69(m, 4H), 7.32(m, 10H), 7.20(m, 4H), 5.23(m, 2H), 5.07(s, 4H), 4.62(m, 2H), 4.16(m, 4H), 3.72(d, J=11.1Hz, 3H), 3.14(m, 4H), 0.94(m, 4H), 0.026(s, 18H); IR (neat, cm^{-1}) 2954, 2339, 1747, 1252, 1039, 860.

L-Phenylalanine, 4-[4-[[2-[(1,1-dimethylethoxy)carbonyl]amino]-3-methoxy-3-oxopropyl]phenyl]methoxyphosphinyl-N-[(phenylmethoxy)carbonyl]-2,2-(trimethylsilyl)ethyl ester, stereoisomer 9

Phosphinic acid anhydrous prepared from 50% aqueous solution (procedure is in PAPER I) (28.4 mg, 0.43 mmol) was weighed carefully and mixed with trimethyl orthoformate (228 mg, 2.15 mmol) in a vial under N_2.

The mixture was kept at room temperature under N_2 for 1 hour whereupon the formation of methyl phosphinate was complete. N-Boc-iodophenylalanine methyl ester **5** (64.9 mg, 0.14 mmol), bis(triphenyl phosphine)palladium chloride (5.0 mg, 0.0072 mmol) and propylene oxide (87 mg, 1.5 mmol) were placed in a flask and dissolved in 0.3 ml CH_3CN. The flask was fitted with a condenser. The whole system was evacuated and filled with N_2. Methyl phosphinate solution was transferred into the flask by syringe. The reaction mixture was heated to reflux in a ~90°C oil bath for 4 hours. The reaction was followed to completion by TLC (EtOAc, product R_f=0.35, S.M. R_f=0.91). The reaction mixture was diluted with about 10ml EtOAc and washed with $NaHCO_3$ (satd.) 5 x15ml, H_2O and NaCl (satd.). The EtOAc layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. This crude product was ready to be carried on to the next step without any purification. For the characterization, this compound was purified by flash chromatography (EtOAc). ^1H NMR (300 MHz, $CDCl_3$) δ 7.71(m, 4H), 7.33(m, 5H), 7.23(m, 4H), 5.27(m, 1H), 5.08(s, 2H), 5.00(m, 2H), 4.61(m, 2H), 4.19(dd, J=17.4, 8.4Hz, 2H), 3.74(d, J=11.4Hz, 3H), 3.12(m, 4H), 1.40(s, 9H), 0.95(t, J=8.1Hz, 2H), 0.031(s, 9H); ^{13}C NMR (75.43 MHz, $CDCl_3$) δ 171.93, 171.31, 155.58, 154.96, 140.95, 140.76, 136.15, 131.98, 131.84, 129.57(d, J=137.3Hz), 129.50(d, J=138.8Hz), 129.78(d, J=1.5Hz), 129.59(d, J=1.5Hz), 128.57, 128.28, 128.13, 80.15, 77.27, 67.06, 64.21, 54.65, 54.17, 52.40, 51.61(d, J=6.0Hz), 38.42, 38.20, 28.28, 17.41, -1.50; ^{31}P NMR (121.42 MHz, $CDCl_3$) δ 33.62; IR (NaCl, cm^{-1}) 3273, 2951, 1718, 1526, 1366, 1215, 1032.

N-benzyloxycarbonyl-4-iodo-L-phenylalanine trimethylsilylethyl ester **3** (60.1 mg, 0.114 mmol) and tetrakis(triphenylphosphine) palladium(12.8 mg,

0.0114 mmol) were placed in the flask containing the crude monophenylalanyl phosphinate. Propylene oxide (87 mg,1.5 mmol) and 1.0 ml CH_3CN were added into the flask. The flask was sealed under N_2. The brown solution was refluxed in a ~85°C oil bath under N_2 for 4 hours. The reaction was followed by TLC (EtOAc, product R_f= 0.56, S.M. R_f=0.35). Solvent was rotary evaporated. Crude product 9 was purified by flash chromatography twice (3:7 CH_3CN:CH_2Cl_2 and 75:15 EtOAc:Hexanes). The purified product was obtained in 54% yield (46mg). ^1H NMR (300 MHz, $CDCl_3$) δ 7.71(m, 4H), 7.33(m, 5H), 7.23(m,4H), 5.27(m, 1H), 5.08(s,2H), 5.00(m, 1H), 4.61(m, 2H), 4.19(dd, J=8.4, 17.4Hz, 2H), 3.74(d, J=11.7Hz, 3H), 3.70(s, 3H), 3.12(m, 4H), 1.40(s, 9H), 0.95(t, 2H), 0.031(s,9H); ^{13}C NMR (75 MHZ, $CDCl_3$) δ 171.93, 171.31, 155.58, 154.96, 140.95, 140.76, 136.15, 131.98, 131.84, 129.57(d, J=137.3Hz), 129.78(d, J=1.5Hz), 129.59(d, J=1.5Hz), 128.57, 128.28, 128.13, 80.15, 77.27, 67.06, 64.21, 54.65, 54.17, 52.40, 51.61, 38.42, 38.20, 28.28, 17.41, -1.50; IR(NaCl, cm^{-1}) 3273, 2951, 1718, 1526, 1366, 1215, 1032; mass for $C_{38}H_{51}N_2O_{10}SiP$, observed (M+1)755.6, ($M+NH_4$) 772.6.

L-Phenylalanine, 4-[4-[[2-[(1,1-dimethylethoxy)carbonyl]amino]-3-methoxy-3-oxopropyl]phenyl]methoxyphosphinyl-N-[(phenylmethoxy)carbonyl]-2,2-(trimethylsilyl)ethyl ester, stereoisomer 10

N-Cbz-iodophenylalanine (3.03 g, 7.13 mmol) was mixed with 20ml H_2O. Aqueous K_2CO_3 solution (1.11 g, 8 mmol, 20%) was added dropwise. The cloudy mixture turned to clear solution when the pH was close to 7. This solution was rotary evaporated. White solid recedue was dissolved in 10ml

DMF and treated with benzylchloride (911 mg, 7.2 mmol) and potassium iodide (12 mg, 0.072 mmol). The mixture was stirred at 50°C-60°C for 1 day till starting material despaired on TLC (CH_3CN, product R_f=0.63, S.M. R_f=0). HO was added to the mixture to dissolve inorganic salts. The mixture was rotary evaporated at 50°C (H_2O bath). Residue was extracted with CH_2Cl 2and H_2O. CH_2Cl_2 layer was washed with H_2O 4 times and satd' NaCl The CH_2Cl_2 solutin was driewd over Na_2SO_4. After removal of solvent, crude product N-Cbz-iodophenylalanine benzyl ester was purified by flash chromatography (4:6 EtOAc:hexanes). Purified product was obtained in 58% yield as white crystal (2.05 g). ^1H NMR (300 MHz, CDC_3) δ 7.49(d, J=8.1Hz, 2H), 7.36(m, 10H), 6.70(d, J=8.1Hz, 2H), 5.20(m, 1H), 5.11(m, 4H), 5.05(m, 1H), 3.03(m, 2H).

Phosphinic acid anhydrous (preparation procedure is in PAPER I) (40.3 mg, 0.61 mmol) was weighed carefully and mixed with trimethyl orthoformate (324 mg, 3.05 mmol) in a vial under N_2. The mixture was kept at room temperature under N_2 for 1 hour whereupon the formation of methyl phosphinate was complete. N-Cbz-iodophenylalanine benzyl ester (100 mg, 0.20 mmol), palladium acetate (2.3 mg, 0.041 mmol) and N-methylmorpholine (21.0 mg, 0.21 mmol) were placed in a 3ml reacti vial and dissolved in 0.8 ml CH_3CN. The whole system was evacuated and filled with N_2. Methyl phosphinate solution was transferred into the vial by syringe. The reaction mixture was heated at 79°C with a heating block for 3 hours. The mixture was filtered through silica gel and washed with CH_3CN. Solvent was rotary evaporated. The crude product was redissolved in 0.8ml CH_3CN and transferred in a reacti vial which contained *t*-Boc iodophenylalanine 2,2-

trimethylsilylethyl ester (82.1 mg, 0.163 mmol), tetrakis(triphenylphosphine) palladium (7.5 mg, 0.0065 mmol) and N-methylmorpholine (21.0 mg, 0.21 mmol). The first step was repeated. The reaction mixture was heated at 79C overnight. Crude product was purified by flash chromatography (7:4 ethyl acetate:hexanes). Product 10 was obtained as colorless oil in 36% yield (48.4 mg). H NMR (300MHz, $CDCl_3$) δ 7.66(m, 4H), 7.34(m, 10H), 7.08(m, 4H), 5.30(m, 1H), 5.11(m, 4H), 5.02(m, 1H), 4.69(m, 1H), 4.56(m, 1H), 4.18(m, 2H), 3.71(d, J=11.1Hz, 3H), 3.14(m, 4H), 1.39(s, 9H), 0.93(m, 2H), 0.03(s, 9H); IR (neat, cm^{-1}) 3335, 2951, 2251, 1811, 1717, 1506, 1456, 1254, 1159, 1020, 858, 733.

Dipeptide 14

L-Phenylalanine, 4-[4-[3-methoxy-3-oxopropyl][2-ammonium trifluoroacetate]phenyl]methoxyphosphinyl-N-[(phenylmethoxy)carbonyl]-2,2-(trimethylsilyl)ethyl ester **15** (921.3 mg, 1.2 mmol) and L-Phenylalanine, 4-[4-[[2-[(1,1-dimethylethoxy)carbonyl]amino]-3-methoxy-3-oxopropyl]phenyl] methoxyphosphinyl-N-[(phenylmethoxy) carbonyl]carboxylic acid **16** (803.9 mg, 1.23 mmol) were dissolved in 4.0 ml DMF. HBTU (780 mg, 2.02 mmol) was added to the DMF solution. Finally, N-methylmorpholine (263 mg, 2.6 mmol) was added. The reaction mixture was stirred at room temperature under N_2 for 1.5 hours. Product was checked by TLC (85:15 CH_3CN:EtOH, Product R_f= 0.7, S.M. R_f=0). The reaction was stopped when the amine was consumed (ninhydrin negative). The mixture was diluted with 20 ml EtOAc. The EtOAc solution was washed with $NaHCO_3$ (satd.), citric acid (50 mM), H_2O and NaCl (satd.). The organic layer was dried with Na_2SO_4. Na_2SO_4 was removed by

filtration. Crude product was obtained by removal of solvent from the filtrate by rotary evaporation. Product was purified by flash chromatography (1:1 acetone:CH_2Cl_2). Purified product **14** was obtained in 77% yield (1.12 g) as a colorless oil. ^1H NMR (300 MHz, $CDCl_3$) δ 7.69(m, 8H), 7.32(m, 10H), 7.22(m, 8H), 6.65(m, 1H), 5.47(m, 1H), 5.37(m, 1H), 5.06(s, 1H), 5.04(s, 1H), 4.78(m, 1H), 4.64(m, 1H), 4.42(m, 1H), 4.19(m, 2H), 3.68(m, 6H), 3.69(s, 6H), 3.10(m, 8H), 1.44(s, 9H), 0.95(m, 2H), 0.028(s, 9H); ^{31}P NMR (121.42 MHz, $CDCl_3$) δ 33.56; IR (NaCl, cm^{-1}) 3254, 3038, 2953, 1717, 1529, 1445, 1252, 1217, 1036, 733.

L-Phenylalanine, 4-[4-[3-methoxy-3-oxopropyl][2-ammonium trifluoroacetate]phenyl]methoxyphosphinyl-N-[(phenylmethoxy)carbonyl]-2,2-(trimethylsilyl)ethyl ester 15

Bisphenylalanylphosphinate **9** (35.3 mg, 0.0468 mmol) in 2 ml 5% TFA/CH_2Cl_2 was stirred at RT for 1 hour and followed by TLC (16:3:2.5 MEK:Et_3N:H_2O, product R_f=0.63, S.M. R_f=0.92). The solution was rotary evaporated to dryness. The residue was dissolved in 5 ml EtOAc, washed with H_2O, then dried over Na_2SO_4. Solvent was removed under reduced pressure. The amino acid TFA salt was obtained as colorless oil in 100% yield (35.9 mg). ^1H NMR (300 MHz, $CDCl_3$) δ 8.03(m, 2H), 7.70(m, 4H), 7.30(m, 9H), 5.46(m, 1H), 5.08(m, 2H), 4.63(m, 1H), 4.33(m, 1H), 4.18(m, 2H), 3.78(m, 6H), 3.24(m, 4H), 0.93(m, 2H), 0.028(s, 9H).

L-Phenylalanine, 4-[4-[[2-[(1,1-dimethylethoxy)carbonyl]amino]-3-methoxy-3-oxopropyl]phenyl]methoxyphosphinyl-N-[(phenylmethoxy)carbonyl]carboxylic acid 16

Unsymmetrical bisphenylalanyl phosphinate **9** (71.8 mg, 0.095 mmol) was dissolved in 0.4 ml DMF. 1N tetrabutylammonium fluoride/THF solution (0.24 ml, 0.24 mmol) was added. This mixture was stirred at RT for 30 min and followed by TLC (16:3:2.5 MEK:Et_3N:H_2O, product R_f=0.46, S.M. R_f=0.92) until starting material was comsumed. H_2O and EtOAc were added to the reaction mixture with stirring. The mixture was acidified with 2N HCl to pH 3. Product was extracted with EtOAc. The EtOAc layer was washed with H_2O 4 times and NaCl (satd.), then dried over Na_2SO_4. After removal of solvent and evacuation to dryness, product was obtained as a colorless oil (59.3 mg, 95%). ^1H NMR (300 MHz, $CDCl_3$) δ 7.65(m, 4H), 7.28(m, 9H), 5.46(m, 1H), 5.11(m, 1H), 4.99(m, 2H), 4.72(m, 1H), 4.60(m, 1H), 3.71(dd, J=15.9, 11.1Hz, 3H), 3.70(s, 3H), 3.10(m, 4H), 1.37(s, 9H); ^{31}P NMR (121.42 MHz, $CDCl_3$) δ 35.76, 35.58; IR (neat, cm^{-1}) 3330, 2951, 2925, 2341, 1732, 1171, 1033.

Peptide 19

N-Cbz iodophenylalanine (30.0 mg, 0.071 mmol) and hydroxybenzotriazole (9.6 mg, 0.071 mmol) were dissolved in 0.5 ml THF. This solution was cooled in ice bath. DCC was added to the cold solution. White precipitate was formed. This mixture was stirred for 2h. 4,4-(methoxyphosphinylidene)-L-phenylalamine dimethyl ester diammonium trifluoroacetate salt (28.2 mg, 0.043 mmol) in 0.1ml THF and N-

methylmorpholine (10.1 mg, 0.1 mmol) was added to the active ester mixture. This mixture was stirred at RT overnight. The reaction was checked by TLC (EtOAc, product R_f=0.71, S.M. R_f=0). The precipitation was filtered out and filtrate was rotary evaporated. Residue was dissolved in EtOAc and washed with $NaHCO_3$ (satd), citric acid (50mM), H_2 and NaCl (satd), dried over Na_2SO_4. EtOAc was rotary evaporated. Crude peptide was purified by flash chromatography (8:2 EtOAc:hexanes). Product was obtained in 32% yield as colorless oil (16.9 mg). ^1H MNR(300 MHz, $CDCl_3$) δ 7.62(m, 4H), 7.53(m, 4H), 7.29(m,10H), 7.09(m, 4H), 6.85(m, 4H), 6.52(m, 2H), 5.45(m, 2H), 5.04(m, 4H), 4.77(m, 2H), 4.36(m, 2H), 3.68(m,9H), 2.98(m, 8H).

Macrocyclic Peptide 28

The dipeptide **14** (774 mg, 0.60 mmol) was dissolved in 1ml DMF and treated with 1N tetra-*n*-butyl ammonium fluoride/THF solution (1.48 ml, 1.48 mmol). The yellow solution was stirred at room temperature for 1 hour. The reaction was followed by TLC (16:3:2.5 MEK:AcOH:H_2O product R_f=0.59, S.M. R_f=0.91). H_2O and EtOAc were added to the reaction mixture and stirred. The pH of the mixture was adjusted to 3 by adding 0.2N HCl. Product was extracted with EtOAc. EtOAc solution was washed with citric acid(50 mM) 3 x15ml, H_2O and NaCl (satd.). The EtOAc layer was dried over Na_2SO_4, filtered, and solvent was rotary evaporated under reduced pressure. The product was obtained as a colorless oil (694 mg, 97%). The crude product was carried on to the next step without further purification. ^1H NMR (300 MHz, $CDCl_3$) δ 7.61(m, 8H), 7.25(m, 18H), 5.91(m, 1H), 5.53(m, 1H), 5.03(m,

6H), 4.83(m, 2H), 4.60(m, 2H), 3.65(m, 6H), 3.69(s, 6H), 3.01(m, 8H), 1.43(s, 9H).

The dipeptide free acid (415 mg, 0.349 mmol) was dissolved in 0.5 ml dried pyridine and evaporated under reduced pressure to remove water. The dried dipeptide acid was sealed in a flask under N_2. THF (0.8 ml, freshly distd.) and pyridine (120 mg, 1.5 mmol, freshly distd.) were added to the flask. Finally, pentafluorophenyl trifluoroacetate (293 mg, 1.05 mmol) was added to the mixture. The reaction solution was stirred at room temperature under N_2 until completion (<30 min). The reaction was followed by TLC (6:4 acetone:CH_2Cl_2, product R_f=0.46, S.M. R_f=0). The reaction mixture was rotary evaporated and vacuum dried. Crude product was purified by flash column chromatography (6:4 acetone:CH_2Cl_2). Purified product **30** was obtained as a colorless oil in 65% yield (0.308 g). ^1H NMR (300 MHz, $CDCl_3$) δ 7.76(m, 8H), 7.31(m,10H), 7.15(m, 8H), 6.79(m, 1H), 5.75(m,1H), 5.66(m, 2H), 5.06(s, 4H), 4.94(m, 1H), 4.75(m, 1H), 4.56(m, 1H), 4.40(m, 1H), 3.65(m, 12H), 3.11(m, 8H), 1.37(s, 9H); IR(NaCl, cm^{-1}) 3279, 2934, 1717, 1533, 1518, 1215, 1016.

The dipeptide pentafluorophenyl ester **30** (340 mg, 0.251mmol) was treated with 3 ml 30% TFA /CH_2Cl_2 solution. The solution was stirred at room temperature and followed by TLC (16:3:2.5 MEK:AcOH:H_2O, product R_f=0.82, ninhydrin positive, S.M. R_f=0.91) until completion (about 1.5 hours). The solution was rotary evaporated and vacuum dried. The yield was 100% (343 mg). ^1H NMR (300 MHz, $CDCl_3$) δ 9.81(m, 2H), 7.68(m, 8H), 7.28(m, 18H), 6.63(m, 1H), 5.91(m, 1H), 5.43(, 1H), 5.11(s, 2H), 5.03(s, 2H), 4.78(m, 2H), 4.48(m, 1H), 4.34(m, 1H), 3.79(s, 6H), 3.70(m, 6H), 3.14(m, 8H).

DMF (peptide synthesis grade, 300 ml) and hydroxybenzyltriazole (34 mg, 0.251mmol) were stirred with activated 3Å molecular seives at room temperature for 3 hours. This solution was filtered. Fresh distilled diisopropylethylamine (130 mg, 1 mmol) was added into this DMF solution. The flask was sealed under N_2.

The precursor **30** (343 mg, 0.251mmol) in 13 ml DMF was added at RT via syringe pump over 22 hours to the HBT/DMF solution. The addition was slow enough that reaction remained ninhydrin negative throughout. The reaction was followed by TLC (acetone, product R_f=0.66, S.M. R_f=0). The addition was slow enough that reaction remained ninhydrin negative throughout. When the addition finished, DMF was removed by rotary evaporator which was connected to a vacuum pump with a 40°C water bath. The yellow residue was redissolved in 15 ml CH_2Cl_2, and washed with citric acid (50 mM) 2x20 ml, $NaHCO_3$ (satd.) 2x20 ml H_2O and NaCl(satd.). The CH_2Cl_2 solution was dried over Na_2SO_4, filtered and evaporated. Crude product was purified by flash chromatography (85:15 acetone: isopropanol). Purified product **28** was obtained in 38 % yield(103 mg). ^1H NMR (300 MHz, $CDCl_3$) δ 7.62(m, 8H), 7.30(m,10H), 7.06(m,1H), 6.85(m, 1H), 6.50(m,1H), 5.55(m,1H), 5.40(m, 1H), 5.00(s, 4H), 4.81(m, 2H), 4.29(m, 2H), 3.66(m, 12H), 2.88(m, 8H); ^{31}P NMR (121.421 MHz, $CDCl_3$) δ 32.62, 32.42, 32.28; IR (NaCl, cm^{-1}) 3267, 2953, 1744, 1726, 1680, 1564, 1217, 1130, 1036; FAB-Mass (M+1) 1073.

Macrocyclic Phosphinic Acid 34

The macrocycle **32** (10.3 mg, 0.00961 mmol) and sodium iodide (1.6mg, 0.0106 mmol) in 0.2 ml CH_2Cl_2 and 0.1 ml CH_3CN were treated with

trimethylsilyl chloride (1.10mg, 0.0106mmol). The mixture was stirred under N_2 at about 50°C for 2 hours. The reaction was followed by TLC (16:3:2.5 MEK:HOAc:H_2O, product R=0.66, S.M. R=0.86). When the reaction was complete, the mixture was rotary evaporated till dryness. The residue was treated with MeOH and filtered. The MeOH solution was rotary evaporated again. Yellow oil (8.70mg) was obtained (crude yield 88%) as crude product **34**.

This crude product was run through a C-18 flash column and eluted with MeOH:H_2O 1:1 20ml, 6:4 20ml and 7:3 20ml. Colorless oil was obtained and further purified by HPLC: C-18 analytical column; l=210nm; ATT=6-10; CHSP=0.1cm min^{-1}; detector range=+0.99 to - 0.1; flow rate= 0.5ml min^{-1}; eluant=CH_3CN: potassium phosphate buffer (1mM, pH 7); gradient: CH_3CN 20% to 40%, 30min, 40% to 40% 10min; sample injection=about 0.1mg (sample dissolved in 1mM phosphate buffer). Product retention time t_R=14.47 min. This fraction was collected. Solvents were removed. The mixture of phosphinic acid dipotassium salt and phosphate salt was obtained as white solid. ^1H NMR (300MHz, D_2O) δ 7.75(m, 8H), 7.45(m, 10H), 7.19(m, 8H), 6.75(m, 2H), 4.93(m, 2H), 4.87(m, 4H), 4.74(m, 4H), 3.73(m, 6H), 2.91(m, 8H); ^{31}P NMR (121.42MHz, D_2O) δ 21.980, 1.59 (phosphate); FAB-mass ($M+H^+$) 1043.6, ($M+K^+$) 1081.3.

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PAPER III. MOLECULAR RECOGNITION ASSAY

INTRODUCTION

Measurement of binding is an important part of molecular recognition. Common methods used in binding study involve monitoring the change in some physical properties during the complexation of either ligands or substrates such as UV absorption, fluorescence, NMR chemical shift or solubility. Usually these methods require a large amount of ligands and substrates, time and instruments for titration. Some efficient assays have been developed, such as Still's method.[1] This method is carried out by chemical bonding the ligand to a polyacrylamide resin and adding a mixture of potential substrates to the resin. The decrease in bulk solution concentration of those substrates that bind to the resin-bound ligand can be observed by using HPLC before and after addition of the resin. Association constants can be determined by measuring the concentration change. In 1992 Whitesides reported using affinity capillary electrophoresis (ACE) in the measurement of binding constants involving low molecular weight receptors.[2] Vancomycin and N-acyl-alanyl-alanines were chosen as a model system in their study. Binding constants can be determined by two experiments. In the first experiment, they included a range of concentration of negatively charged peptides in the buffer and measured the corresponding change in the mobility of vancomycin which is due to the change in charge of vancomycin on binding the peptide. In the second experiment, they observed the mobility of the ligands and varied the concentraton of vancomycin. By measuring the appearance time of the peak due to vancomycin or to peptides as a function of the concentration of additive in the buffer, binding constants were calculated.

Our efforts in molecular recognition are directed toward peptide-like molecules which are rapidly synthesized, and whose structures may easily be perturbed slightly for optimization of binding selectivity. In connection with this work, a method is needed to rapidly screen many substrates for binding to a specific ligand. We have developed such an assay, which promises to identify those components of a mixture which specifically bind to two-dimensional thin layer chromatography (2D-TLC).[3] A mixture containing the potential binding substrates is applied to the lower left of a reverse phase TLC plate, and eluted in the vertical direction on the first of two domains of equal polarity. After drying the TLC plate, a second elution, perpendicular to the first, carries the substrates onto the second domain, which is coated with the ligand. A specific association between the substrate and the ligand will retard only the second migration of that specific substrate. This compound is thus recognizable as an off-diagonal deviation of the spot. (Figure 1). In this way the retention by normal adsorption modes may be distinguished from a more specific association, even if the association is significantly weaker than is generally useful for affinity chromatography.

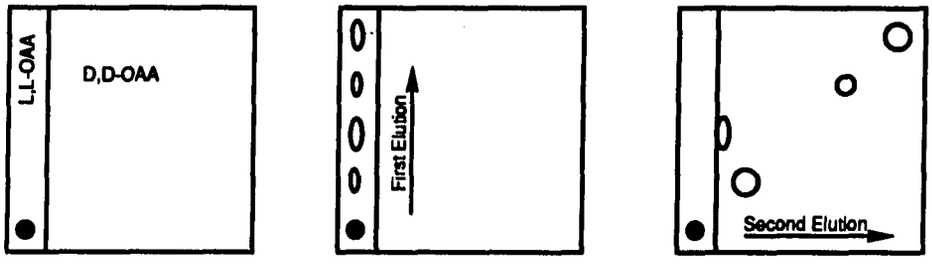
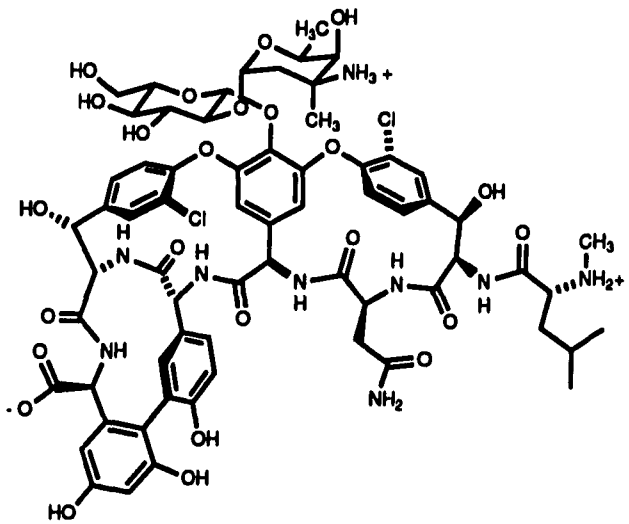


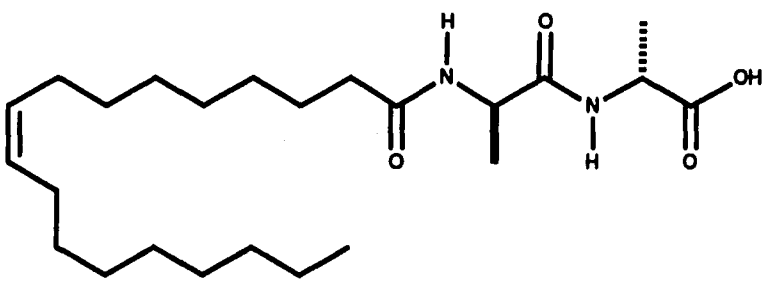
Figure 1. 2D-TLC

RESULTS AND DISCUSSION

Vancomycin was chosen as the binding substrate for the demonstration of this 2D-TLC assay. It is known that vancomycin binds acylated D-alanyl-D-alanines, but not the analogous L-alanyl compounds, in aqueous solution with dissociation constants in the range of 10^{-3} - 10^{-6} M.[4,5]



Vancomycin

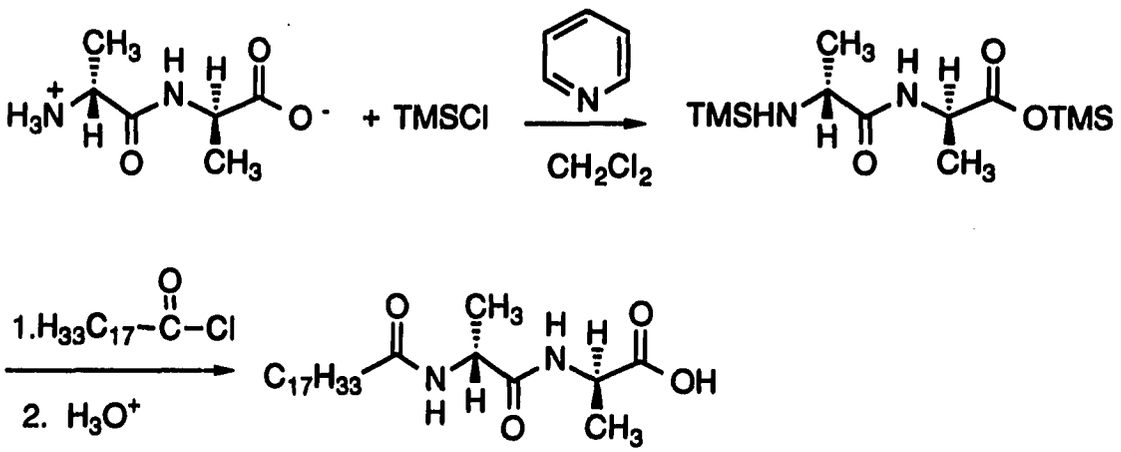


Oleoyl-D-alanyl-D-alanine

Preparation of the Ligand

As a binding ligand for vancomycin, oleoyl-D-alanyl-D-alanine was chosen because it has a binding group, D-alanyl-D-alanine and a long alkyl chain that is similar to the C-18 group on the reverse phase TLC plate. Oleoyl-L-alanyl-L-alanine was used as the stationary phase for the nonbinding domain. Both compounds were made by the same procedure. The synthesis of oleoyl-D-alanyl-D-alanine is shown in Scheme 1. Usually amino acid can be acylated by treating with acid chloride in aqueous solution in the present of NaOH.[6] In the case of amino acid bearing a hydrophobic substituent, diacylation gives an anhydride that leads to epimerization and also multiple coupling. The carboxylic acid group should be protected before the acylation. We used a trimethylsilyl group as the acid protecting group which can be easily installed and removed. The treatment of commercially available D-alanyl-D-alanine with chlorotrimethylsilane in the presence of pyridine gave a doubly silylated dipeptide which then dissolved in CH_2Cl_2. When this dipeptide CH_2Cl_2 solution was treated with oleoyl chloride, only the amino group was acylated because of its higher nucleophilicity than that of trimethylsilyl ester. The product oleoyl-D-alanyl-D-alanine trimethylsilyl ester underwent hydrolysis in acidic aqueous solution upon workup to generate oleoyl-D-alanyl-D-alanine as the desired product in 98% yield. Oleoyl-D,L-alanyl-D,L-alanine was also prepared under the same conditions. No evidence of epimeric oleoyl-alanyl-alanine was detected in the product of oleoyl-D-alanyl-D-alanine with 300 MHz ^1H NMR. We could have readily detected 5% epimer. This reaction procedure gives oleoyl-D-alanyl-D-alanine in high yield which contains a small amount of unreacted oleic acid as impurity. The oleic acid can be easily

removed by flash chromatography. In the later 2D-TLC procedure, oleoyl-D-alanyl-D-alanine would be diluted with oleic acid. Therefore, no purification procedure was applied to the oleoyl-D-alanyl-D-alanine.



Scheme 1.

Preparation of the Stationary Phase

To carry out the assay the ligands are needed to be affixed to the chromatography support. we found that simple adsorption of thte hydrophobic ligands to C-18 plates would suffice. The ligand oleoyl-D-alanyl-D-alanine and its L analog were coated on the reverse phase TLC plate separately by a normal TLC elution method. In order to coat the TLC plate evenly with the ligand, the plate was eluted with a solution of the ligand. The solvent was chosen for this elution was one in which the ligands were eluted with an R_f=1. This ensures the TLC plate was coated with the ligand evenly. Two isomers of oleoyl alanyl alanine can be eluted with R_f=1 on the C-18 plate in a mixed solvent of acetone and chloroform (7:3). This elution method is better than simply dipping the TLC plate into the ligand solution because it coats the plate

evenly and uses the ligands efficiently. The TLC plates coated in this method worked very well in the assay.

The TLC stationary phase was made by eluting Whatman KC18F reverse phase TLC plates in the above coating solution, followed by air drying. Oleoyl -L-alanyl-L-alanine was coated on the left side of the square TLC plate to form a narrow nonbinding domain. Oleoyl-D-alanyl-D-alanine was coated on the remaining area to form a wide binding domain. This TLC assay requires elution twice. There might be some salts formed on the stationary phase since the eluant was the mixture of CH_3CN and aqueous buffer solution. So the L domain was prepared first so that the D domain would be fresh for binding vancomycin. After applying sample on this domain and the first elution, the TLC plate was dried completely. Then the plate was coated with oleoyl-D-alanyl-D-alanine and the second elution was performed.

Eluant

Since the TLC plates are coated with oleoyl alanyl alanine merely by adsorption, the TLC eluant is restricted to the solvents in which the coating compound has $R_f=0$. The eluant should also be able to move vancomycin from the base line and separate each component of a mixture we have chosen for the demonstration of this assay.

Mixtures of CH_3CN and different aqueous buffer solutions, such as phosphate buffer (50mM, pH=7), acetic acid/pyridine buffer (50mM, pH=5.3), ammonium bicarbonate buffer (100mM, pH=8.4), 2-(4-morpholino)-ethane sulfonic acid buffer (0.5mM, pH=5.9) and H_2O have been tested as eluants. All of them are compatible with the TLC stationary phase and are able to separate

vancomycin and a mixture of amino acid derivatives to some extent, but they do not always give well shaped TLC spots. The best eluant found is a combined mixture of 30% acetonitrile and 70% acetic acid/pyridine buffer (1:1, 50mM, pH=5.3).

Amount of Sample Applied to the TLC Plate

For the demonstration of this TLC assay, vancomycin (0.43mM), histidine methyl ester (14mM), methionine methyl ester (14mM), homocysteine thiolactone (14mM) and L-alanine (28mM) was used as analytes. These compounds were chosen because they have different R_fs from that of vancomycin. The volume of the mixture spotted on the TLC plate was estimated about 0.01μl, so that the amount of each component loaded was about 10^{-10} mol. This TLC assay showed good separation and clear spots of the analytes in this low concentration range.

TLC Detection

A solution of 0.1% ninhydrin and 0.5% trifluoroacetic acid in ethanol shows high sensitivity to all components of the mixture and was used to visualize the spots. Ninhydrin is an excellent amino group detecting reagent. It shows different sensitivity in different solvent at different pH. Four ninhydrin solutions have been tested: ninhydrin in pyridine, ninhydrin with phenol in n-butanol, ninhydrin with acetic acid and pyridine in acetone and ninhydrin with trifluoroacetic acid in ethanol. Several other spray solutions have also been tried, such as vanillin/EtOH, ceric sulfate/H_2SO_4, cupric sulfate/H_3PO_4, anisaldehyde/EtOH, 2,4-dinitrophenylhydrozine/EtOH,

potassium permanganate/H_2O. From our experimental results, ninhydrin is the only one that is able to detect all the components in the analytes.

Chromatography

A mixed sample containing 1-5 $x10^{-10}$ M each of histidine methyl ester, vancomycin, methionine methyl ester, homocysteine thiolactone and L-alanine was applied to the narrow oleoyl-L-alanyl-L-alanine coated domain of a square TLC plate, air dried, and eluted on this domain with 7:3 H_2O:CH_3CN buffered to pH 5.3 with 50mM acetic acid/pyridine buffer. The eluant was removed under vacuum, then the large domain was coated with oleoyl-D-alanyl-D-alanine as described above. The second elution was carried out in the same aqueous buffer onto the large domain in a direction perpendicular to the first. Ninhydrin visualization of the spots clearly showed all components on a diagonal line of slope =1, except for the vancomycin spot which remains near the edge of the oleoyl-D-alanyl-D-alanine domain (Table 1). Other compounds we have investigated have all migrated to the diagonal, with the exception of ristocetin, which is also known to have binding properties similar to those of vancomycin.

When oleoyl-alanyl-alanine was directly coated on the C-18 plate, the chromatography did not give good shaped spots. Some analytes showed long retention on the TLC plate. Those problems could be caused by acidic stationary phase. The carboxylic acid group of oleoyl alanyl alanine increases the polarity of the TLC stationary phase and also forms salts with analytes. The acid groups might have strong interactions, such as hydrogen bonding or ion exchange with analytes on the TLC plate and cause irregular shapes of the

spots after the development. In order to solve this problem, oleoyl alanyl alanine potassium salt has been used as the stationary phase. But this method did not improve the shape of the TLC spots. It has been found that when TLC plates were coated with a mixture of oleic acid and oleoyl alanyl alanine, the analytes, such as amino acids and their derivatives, showed spots with good shape. Oleic acid could help to distribute acid groups evenly on the surface of TLC plates. Oleic aicd also functioned like a "buffer", so that changing the concentration of oleoyl alanyl alanine on the plate would not change the acidity and ionic strength of the TLC stationary phase. When the concentration of oleic acid in the coating solution was varied from 25mM to100mM, the resulting TLC plates showed similar results.

Table 1. 2D-TLC data

Conc (mM)	Substance	Distance traveled 1st elution (mm)	R_f (L,L)	Distance traveled 2nd elution (mm)	R_f (D,D)
14	His-OMe	0 - 4	0.04	0 - 3	0.04
50	Vancomycin	7 - 12	0.21	0.5 - 1.5	0.02
14	Met-OMe	13 - 17	0.33	10 - 15	0.30
14	Homocysteine thiolactone	16 - 21	0.40	13 - 19	0.38
28	Ala	37 - 42	0.86	31 - 38	0.82

Note: solvent front: 1st elution: 46mm, 2nd elution: 42mm.

Advantages of this 2D-TLC technique are simplicity and low cost so that it can be used as a general screening method for a large number of compounds. It does not require expensive instruments or quantitative measurements, as most other binding study methods do. Compared to other chromatographic procedures, high resolution separation is not essential in this 2D-TLC method. Even if a mixed sample is not separated completely with the 2D-TLC, an off-diagonal spot is good evidence of special interaction between this component and the ligand. Elution method used to coat the TLC stationary phase provides a simple estimation of ligand surface concentration. Evenly coated TLC plates have the similar surface concentration with the concentration of coating solution. From this approximate surface concentration we can further estimate the binding constants.

In the demonstration of this assay, we used oleoyl-D-alanyl-D-alanine and oleoyl-L-alanyl-L-alanine as two TLC stationary phases which have the equal polarity. However, the equal polarity of two domains is not the essential requirement of this assay. As long as two domains have the similar polarity the assay should be able to show the binding if the complexation occurs between the ligand and the substrate. The simplicity of this technique makes it possible to quickly test many compounds and choose an appropriate one as the nonbinding domain coating material.

This 2D-TLC assay was demonstrated by a simple example, recognition of the binding between vancomycin and D-alanyl-D-alanine. The binding between other ligands and substrates can also be recognized by this assay in the same procedure but under the different TLC conditions. The method is

intended as a first screen to identify, from a large group, those molecules on which to focus study.

EXPERIMENTAL SECTION

Oleoyl-D-alanyl-D-alanine

Treatment of a suspension of D-alanyl-D-alanine (100.9mg, 0.63mmol) in CH_2Cl_2 (5ml) with trimethylsilyl chloride (425.1mg, 3.9mmol) and pyridine (980.8mg, 12.4mmol) gradually caused dissolution of the silylated dipeptide. Upon obtention of a clear solution, oleoyl chloride (180.5mg, 0.60mmol) was added, and the mixture stirred at 23 ^{0}C until acid chloride was consumed (about 4 hours). H_2O and ethyl acetate were added to the reaction mixture. The ethyl acetate layer was washed with 0.5N HCl 3 times and with satd. NaCl. The organic solution was dried over Na_2SO_4, filtered and solvent removed by rotay evaporation. After removal of solvent from the filtrate, product was obtained as white solid in 98 % yield (249.6mg), contaminated with a small amount of oleic acid. The oleic acid could be easily removed by chromatography on silica gel (EtOAc: CCl_4: i-PrOH 1:1:0.2), but was usually left in since the compound was later diluted with oleic acid. ^{1}H NMR (300MHz, $CDCl_3$) δ 8.64(m, 1H), 7.17(d, J=7.2Hz, 1H), 6.48(d, J=7.8Hz, 1H), 5.34(m, 2H), 4.63(m, 1H), 4.54(m, 1H), 2.21(t, J=7.5Hz, 2H), 2.01(m, 4H), 1.61(m, 2H), 1.45(d, J=7.2Hz, 3H), 1.36(d, J=7.2Hz, 3H), 1.28(d, J=7.2Hz, 20H), 0.89(t, J=6.3Hz, 3H).

The synthesis of oleoyl-L-alanyl-L-alanine followed the same procedure.

2D-TLC Procedure

The Whatman KC18F reverse phase TLC plate was eluted with a solution of 10mM oleoyl-L-alanyl-L-alanine and 40mM oleic acid in 70:30:1 CH_3CN:

$CHCl_3$: pyridine. The narrow nonbinding domain was prepared by this elution on the left side of the TLC plate.

A mixture of vancomycin (0.43mM), histidine methyl ester (14mM), methionine methyl ester (14mM), homocysteine thiolactone (14mM) and L-alanine (28mM) in aqueous solution was spotted on the nonbinding domain of the TLC plate and followed by air dry. The TLC plate was eluted in a mixed eluant 7:3 CH_3CN: aqueous buffer (50mM, 1:1 acetic acid: pyridine, pH=5.3). The plate was dried under vacuum after the elution. The second binding domain of the plate was coated with oleoyl-D-alanyl-D-alanine as described above. The second elution of the sample was carried out in the same eluant in a direction perpendicular to the first, onto the large domain. After the second elution and drying, the plate was visualized by spraying a solution of 0.1% ninhydrin and 0.5% trifluoroacetic acid in ethanol, and heating to show spots.

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GENERAL CONCLUSION

A new class of potential binding sites containing arylphosphinic unit has been prepared. With these molecules, an extensive and broad range of study in the area of hydrophobic interaction, ionic effect on the binding and catalysis can be carried out. The complexation of the phenylalanlyphosphinic unit with metal ions and the binding properties of this type of self-assembled cyclic complexes with aromatic guests, the conversion of anionic arylphosphinic acid binding sites to cationic binding sites and the comparison of their binding, the incorporation of arylphosphinic acid units into peptides to generate DNA binding cavities and the application of arylphosphinic acid units in the study of tyrosine phosphotase inhibition to develop anticancer drugs are the research projects carried out in our research lab. More and more promising results have shown the significance of this type of binding sites in molecular recognition and the potential application in organic chemistry. The synthetic procedures developed in the preparation of this class of potential binding sites have provided the useful tools for the further synthetic binding site construction and also for organic synthesis, especially the methods for synthesis of arylphosphinates has received attention from industrial researchers.

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