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Design and synthesis of small-molecule inhibitors as anti-malarial and anti-serpin agents

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Design and Synthesis of Small-Molecule Inhibitors as Anti-malarial and Anti-serpin Agents

by

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ABSTRACT

This work describe the design, synthesis and evaluation of small-molecule inhibitors of two different biological targets: *Plasmodium falciparium*, the most virulent malarial parasite in humans, and plasminogen activator inhibitor-1 (PAI-1), an endogenous serine proteases inhibitor implicated in a wide range of biological processes. Malaria is one of the top global health threats, and there is a great need for developing effective new chemotherapies. PAI-1 is a major component in the regulation of the plasminogen activation system, and the overexpression of this protein has been implicated in a number of conditions, such as thrombosis, atherosclerosis, and myocardial infarction. The research discussed concerns the design and synthesis of enantiomerically pure anti-malarial compounds containing chiral 1,2-aminoalcohol moiety. In addition, the synthesis and biological evaluation of several highly potent, novel, polygalloyl PAI-1 inhibitors based on common carbohydrates and tethers of various lengths is also presented.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

Chapter I

Introduction

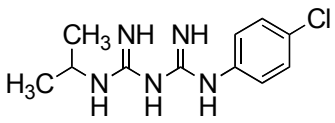
Malaria is an infectious disease caused by a one-celled parasite from the genus *lasmodium.* Four species of *Plasmodium* are capable of infecting humans: *P. falciparum, P. vivax, P. malariae and P. ovale*. *Plasmodium falciparum* is responsible for the most malaria deaths in humans, especially in Africa. There are about 300 to 500 million new cases of malaria every year, and more than one millions deaths.[1]

The malarial parasites are generally transmitted to humans by the bite of the female *Anopheles* mosquito. Other modes of transmission are blood transfusion, needle stick injury, or an infected mother to a growing fetus. When an infected *Anopheles* mosquito bites a human, it takes in blood and injects saliva that contains an infectious form of the parasite, the sporozoites, into a person's bloodstream. The sporozoites invade liver cells and develop into schizonts, each of which contains thousands of merozoites (another stage of the parasite). When schizonts reach maturity, they rupture and release the merozoites back into the bloodstream, where they invade the host's red blood cells. In these cells, the growing parasite consumes and degrades hemoglobin into individual amino acids. The malarial parasites then use these amino acids for protein synthesis and growth, because the parasite has limited capacity for *de novo* synthesis of amino acids.[2] Degradation of hemoglobin causes the release of the iron-containing heme, which is toxic to the parasite at increased levels, so the parasite detoxifies it by converting into an insoluble polymeric pigment, called hemozoin.[3] Merozoites in the red blood cells undergo an asexual reproduction, forming another generation of schizonts, filled with more merozoites. The same cycle repeats as these merozoites are released into the

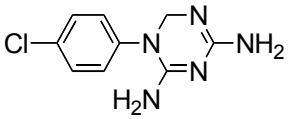
bloodstream, invading uninfected red blood cells. As this asexual cycle repeats several times, some of the merozoites in the blood stream progress through a sexual cycle and develop into either male or female gametocytes. When gametocytes reach maturity, they are released into the circulatory system.[4,5] This sexual stage is responsible for the infection of the *Anopheles* mosquito and subsequent transmission of the parasite to the next person.

Treatment of malaria in humans

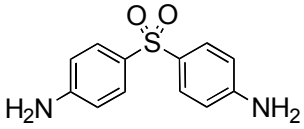
New, effective treatments for malaria are urgently needed, *as P. falciparum* has developed some level of resistance to nearly all currently available drugs.[6] The most common treatment of malaria is based on two main classes of compounds: antifolates and quinoline-containing compounds. The antifolates include proguanil (**I-1**) and dapsone (**I-3)**, while the quinolines include chloroquine (**I-4**) and mefloquine (**I-5**).



I-1. Proguanil

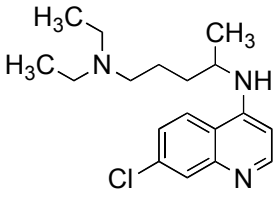


I-2. Cycloguanil

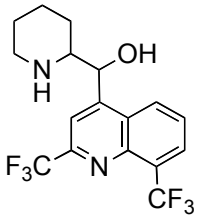


I-3. Dapsone

Figure I-1.- Available antifolates for treatment of malaria



I-4. Chloroquinone

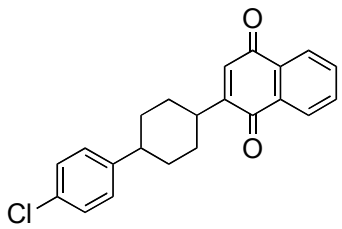


I-5. Mefloquine

Figure I-2.- Available quinolines for treatment of malaria

Proguanil **I-1** is metabolized in the body by cytochrome P450 to the active metabolyte, cycloguanil. Cycloguanil then inhibits plasmodial dihydrofolate reductase,[7] an enzyme responsible for the *de novo* synthesis of folate. Inhibition of this metabolic pathway leads to the inhibition of the parasites' ability to synthesize pyrimidines, purines and some amino acids synthesis.[3] Proguanil is used as an antimalarial treatment in combination with atovaquone **I-6** because resistance of *P. falciparum* to proguanil developed relatively rapidly.[8]

Dapsone **I-3** inhibits plasmodial dihydropteroate synthetase. Dihydropteroate synthetase is an enzyme responsible for the synthesis of dihydrofolic acid.[9] Like proguanil, dapsone is used in combination with another *P. falciparium* inhibitor, chloroquine.[9]



I-6. Atovaquone

Figure I-3. Example of a naphthalene for treatment of malaria

Atovaquone **I-6** is a chemical that belongs to a class of inhibitors referred to as naphthalenes. Antimalarial activity of atovaquone consists of disruption of the parasite's electron transport chain, which ultimately prevents the parasite from replicating its DNA.[3,9] Due to high resistance of *P. falciparum* to atovaquone, it is generally used in combination with proguanil.[9]

The development of resistance by *P. falciparium* to antifolates is associated with mutations in the genes encoding dihydropteroate synthetase and dihydrofolate

reductase.[1,9] The level of resistance is directly correlated with the number of mutations in the genes for the two enzymes.[10]

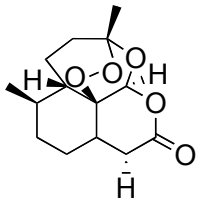
The most common quinoline-based compounds used as antimalarials are chloroquine **I-4** and mefloquine **I-5**. The proposed mechanism of action for chloroquine is that the drug accumulates in the food vacuoles of the parasite where it interferes with the detoxification of heme by polymerization into hemozoin, and the resultant complex is highly toxic to the parasite.[3,9] Chloroquine has for many years been the antimalarial treatment with the most widespread use due to its efficacy, ease of administration, and low cost, but ever-increasing resistance has decreased its usefulness. The mechanism of chloroquine resistance is not well understood, but the assumption is that the problem does not concern resistance to the mechanism of action of the drug, but instead concerns lower concentrations of chloroquin in the food vacuole of the parasite.[3] Many hypotheses have been suggested; some evidence indicates that the resistance to chloroquine is due to the increased drug efflux, while others suggest a decrease in affinity for heme by chloroquine.[3]

Mefloquine **I-5** was developed in the 1970s as resistance of *P. falciparum* to chloroquine, the most common treatment at that time, was increasing dramatically, necessitating the development of new treatments. The mechanism of action of mefloquine is similar to that of chloroquine, but its affinity for heme is lower than that of chloroquine.[3] The mechanism of resistance of *P. falciparum* to mefloquine is associated with mutation or amplification of the P-glycoprotein, encoded by the MDR gene family, which encode ATP-binding structures.[9] Interestingly, some studies have shown that

malarial strains with an increased resistance to mefloquine tend to have increased sensitivity to chloroquine **I-4**.[3]

Treatment of malaria with mefloquine can cause occasionally severe mental disorders, including psychosis, paranoia, suicide, and hallucinations.[11] The mechanisms that induce these side effects are not well understood, but some activities of mefloquine, such as inhibition of P-glycoprotein, interference with calcium homeostasis and gap junction formation in neurons could contribute to mefloquine-induced psychosis.[11]

In addition to the two main classes of antimalarial compounds, antifolates and quinoline-containing compounds, artemisinin derivatives are used to treat multi-drug resistant strains of malaria parasite. Artemisinin **I-7** is a naturally occurring compound, extracted from *Artemisia annua* (the Sweet Wormwood plant), and is present in extracts used as a traditional Chinese medicine to treat fevers.



I-7. Artemisinin

Figure I-4.- Available drug for Malaria treatment

The mode of action of artemisinin is not fully understood, but an intense research effort is currently devoted towards its elucidation. The structure of artemisinin, in particular its peroxide bond, plays an important role in its activity. When the malarial parasite enters the blood cell of the host, it consumes hemoglobin and releases heme. The peroxide bond of artemisinin interacts with the iron in heme, catalyzing the formation of

oxygen free-radicals that damage or kill the parasite.[3] Artemisinin is among the few drugs that have not yet developed resistance by *P. falciparum*. Because of its limited availability and high costs of isolation and administration, treatment with artemisinin is seen as too expensive for poverty stricken populations in Africa and Southeast Asia. In light of this, a more cost-effective drug is clearly needed.

We focused our research on the synthesis and development of antimalarial compounds that contain a 1,2-aminoalcohol moiety. This class of compounds has been proposed to inhibit an essential protease within the malarial parasite. By the inhibition of the target protease, which is required for hemoglobin degradation, the life cycle of the malarial parasite should be disrupted. Howarth[12] and coworkers reported the one-pot synthesis of six 1,2-aminoalcohols that were tested as antimalarials and protease inhibitors. **Figure I-5** illustrates the structure of the six 1,2-aminoalcohols.[12]

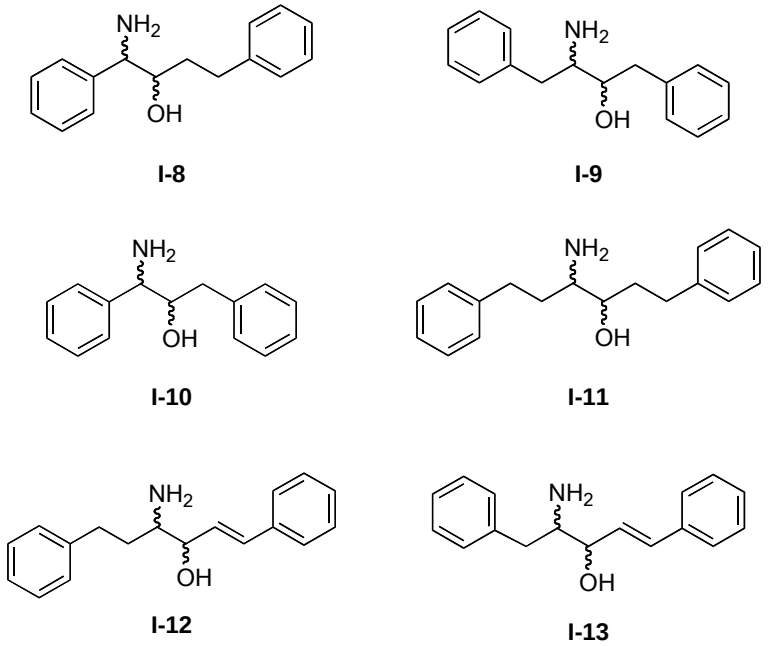


Figure I-5 - 1,2-aminoalcohols synthesized by Howarth and Lloyd[12]

Biological testing of the above compounds was done on two human strains of *P. falciparum* parasite, a chloroquine-resistant strain (W2) and a chloroquine-sensitive strain (D6). Compounds were also tested against leucine aminopeptidase (LAP), a protease essential to hemoglobin degradation. The results summarized in **Table I-1** showed that the most effective inhibitor against the chloroquine resistant W2 strain is compound **I-13**, which has the lowest IC_{50} value.

Table I-1. Activity of 1,2-aminoalcohols against *P. falciparum* and leucine aminopeptidase

	IC_{50} (μM)		
Compound	W2	D6	Effect on LAP
I-8	14.89 +/- 1.17	-	activation
I-9	14.47 +/- 1.04	15.55 +/- 1.24	activation
I-10	11.65 +/- 0.78	-	activation
I-11	9.60 +/- 0.92	-	inhibition
I-12	5.86 +/- 0.59	13.33 +/- 0.84	inhibition
I-13	2.64 +/- 0.25	10.12 +/- 0.69	activation

The activating effect of LAP by compound **I-13** suggests that while it might be an inhibitor of an essential protease, the target enzyme of this compound is different than leucine aminopeptidase. Taking in consideration these promising initial results, it is well worth exploring and taking a closer look at this class of compounds.

1,2-Aminoalcohols are compounds with two chiral centers at the carbon atoms attached to the amino and hydroxyl groups. Howarth and coworkers performed a "one pot" synthesis of the 1,2-aminoalcohols, which provided racemic mixtures containing the

four possible diastereomers, but did not separate them before submitting them to biological testing. Therefore, there is no information regarding the stereochemical preference of the target enzyme for one configuration over the others.

Our plan is to synthesize all four diastereomers separately in a stereoselective fashion and test each of them against the malarial parasite, in order to determine which configuration of the chiral aminoalcohols displays the highest activity.

Results and Discussion

Introduction

1,2-Amino alcohols are an important class of organic compounds with wide biological and chemical applications. This motif is present in many naturally occurring compounds, including the amino acids serine and threonine, and is also common in a wide array of synthetic molecules.[13] It has been previously reported that 1,2-amino alcohols are potential inhibitors of malarial parasite *P. falciparium*, the parasite responsible for most malarial deaths in humans.[12] In this study we have focused on the synthesis of enantiomerically pure 1,2-amino alcohols, with the intention of submitting these compounds for biological testing against *P. falciparium*, and determining the most active stereoisomer against the parasite.

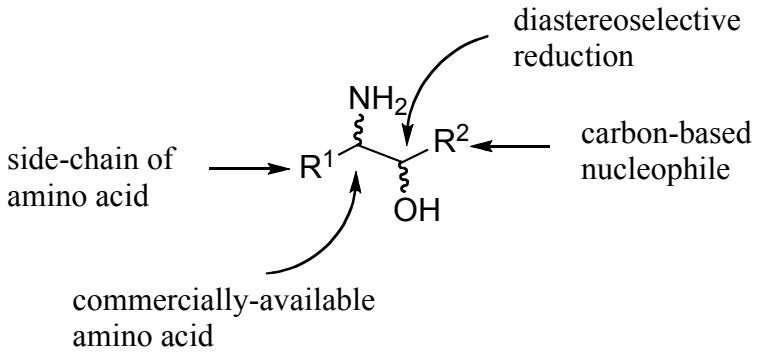
Our approach towards the synthesis of 1,2-amino alcohols is based on using amino acids as affordable, optically pure starting materials (**Scheme I-1**). Starting with D- or L- amino acids, we devised a multistep synthesis to lead us to the final compounds that contain 1,2-amino alcohol moiety. The first step in our methodology was the protection of the amino group with a tert-butoxycarbonyl group (Boc) in order to avoid participation of this group in the next steps of the synthesis. The second step involved preparation of the Weinreb amide, which was then allowed to react with various Grignard reagents. We set the second chiral center by the diastereoselective reduction of the prochiral carbonyl group. Reduction of the ketone with achiral reducing agents ($NaBH_4$, $LiAlH_4$ and DIBAL-H) afforded poor diastereoselectivity, while reduction with chiral agent (R)-Alpine Borane provided high selectivity for one of the diastereomers. The last

step in our synthesis was deprotection of amino group, in which the Boc group was easily removed by treatment with trifluoroacetic acid.

Syntheses of amino alcohols

The proposed five-step synthesis is a versatile method that allows the synthesis of a large variety of compounds that contain an 1,2-amino alcohol moiety with a good yield and high purity. This general strategy for drug synthesis is summarized in **Scheme I-1:**

Scheme I-1



Amino acids (D or L)
R^1: 20 amino acids naturally available

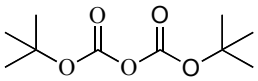
Carbon nucleophile
R^2-M
R^2:alkyl, aryl, phenyl, etc
M: Li, MgBr, MgCl, etc

We initiated the synthesis of vicinal amino alcohols, such as compound **I-13**, with a naturally occurring amino acid, phenylalanine. Phenylalanine possesses a chiral center at the carbon bearing amino group, which makes possible two enantiomers (**Figure I-6.**): naturally occurring L-phenylalanine and the unnatural D-phenylalanine.

a) L-Phenylalanine b) D-Phenylalanine

Figure I-6. Enantiomers of phenylalanine

Tert-butoxycarbonyl (Boc) (**Figure I-7**) was chosen to protect the amino group. The choice was based on the properties of this group, specifically its stability toward nucleophiles and bases, and the relative ease with which it can be removed from the final product. Although N-Boc protected amino acids are acid sensitive, careful selection of proper reaction conditions for the subsequent steps of the synthesis makes the Boc group a good choice.

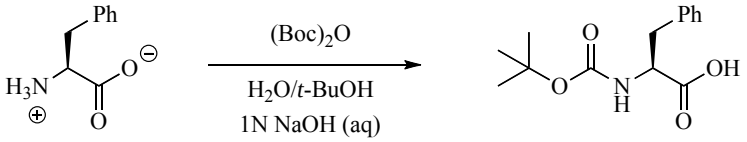


I-14

Figure I-7. $(Boc)_2O$ – Reagent for introduction of *tert*-butoxycarbonyl protecting group

The reaction of Boc_2O with L-phenylalanine (**Scheme I-2**) was done at room temperature in *tert*-butanol as solvent.[14] The N-Boc protected product, 2(*S*)-(*tert*-butoxycarbonylamino)-4-phenylbutanoic acid (**I-15**) was obtained in high yield (96%) and the crude compound was used in the next step without further purification.

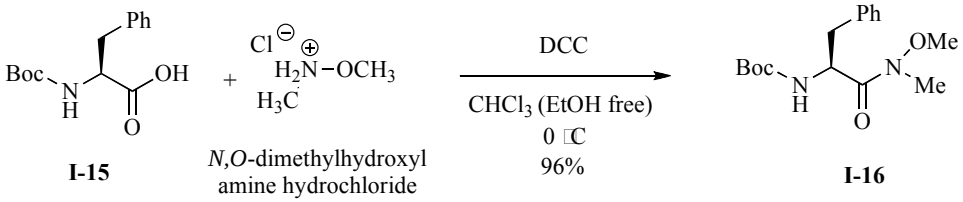
Scheme I-2



L-Phenylalanine **I-15**

Weinreb amides, a specific form of *N*-methoxyamide, are important intermediates in the synthesis of various carbonyl compounds. Weinreb amide **I-16** was synthesized according to a published procedure.[15] Reaction of a 1:1 molar ratio of 2(*S*)-(*tert*-butoxycarbonylamino)-4-phenylbutanoic acid **I-15** and *N,O*-dimethylhydroxylamine hydrochloride, in the presence of an appropriate coupling reagent, was the method used for the synthesis of Weinreb amide **I-16** (**Scheme I-3**).

Scheme I-3



I-15 *N,O*-dimethylhydroxyl amine hydrochloride **I-16**

N,N'-dicyclohexylcarbodiimide, (DCC) (**Figure I-8**) was the reagent used for coupling the carboxyl group of compound **I-16** with the *N*-alkoxyamine for the formation of Weinreb amide. The drawback of using DCC is formation of a urea byproduct, which is very hard to remove from the reaction mixture. Use of ethanol-free chloroform as solvent, however, improved the isolated yield of **I-16** to 96%.

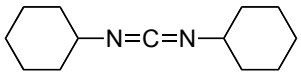


Figure I-8 *N*,*N*-Dicyclohexylcarbodiimide (DCC)

Addition of a hard carbon nucleophile to a carbonyl group is an important method used to form a carbon-carbon bond. The reaction of Grignard reagents with Weinreb amides give the corresponding ketones, generally in good yield, as nucleophilic addition to the carbonyl proceeds to form a stable tetrahedral intermediate that blocks over-addition.[15] In our case, the reaction was run at –78 °C in THF,[16] and the best yield was obtained when 3.3 equivalents of Grignard reagent were allowed to react with 1 equivalent of Weinreb amide (**Table I-2**).

Scheme I-4

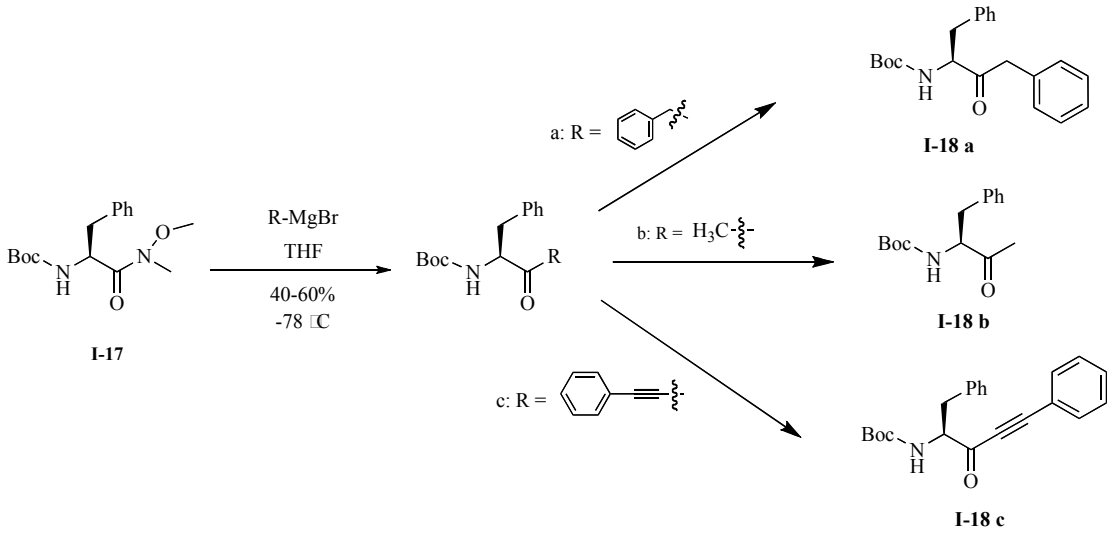
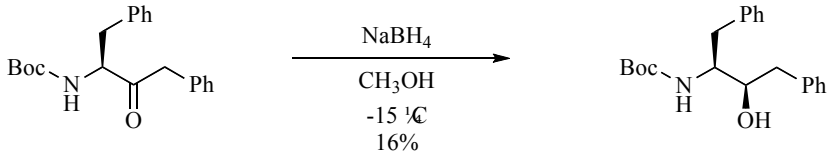


Table I-2. Nucleophilic addition of various Grignard reagents to Weinreb amide **I-17**.

Entry	R-	Molar Equivalents	Product	Yield (%)
1	a	3.3	**I-18a**	46
2	b	3.3	**I-18b**	51
3	c	3.3	**I-18c**	60

Reduction of α-amino ketones is an efficient method to stereoselectively synthesize 1,2-amino alcohols. Sodium borohydride ($NaBH_4$) and lithium aluminum hydride ($LiAlH_4$) are achiral reducing agents capable of reducing a wide range of substrates. Both reagents are sources of hydride ion [H^-], a very strong base and in association with boron and aluminum respectively, a good nucleophile. Reduction of the 2(*S*)-(*tert*-butoxycarbonylamino)-4-phenyl-1-benzyl ketone (**I-18a**) with $NaBH_4$ resulted in the exclusive formation of 1,2-syn-amino alcohol **I-19** (**Scheme I-5**) as indicated by ^1H NMR. Reduction of **I-18a** was carried out in methanol at –15 ºC[16] and afforded the corresponding amino alcohol in 16% yield as a single diastereomer.

Scheme I-5

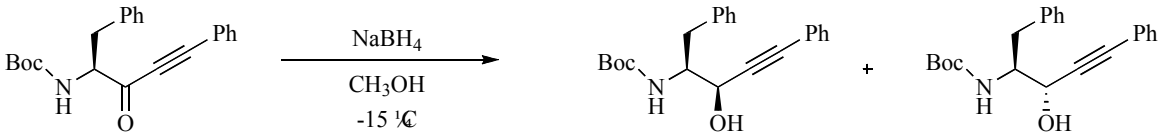


I-18a **I-19**

In the case of unsaturated α-amino ketone **I-18c**, the carbonyl group was more difficult to selectively reduce to the corresponding alcohol because triple bond reduction was a competing reaction.[17]

Reduction of 2(*S*)-(*tert*-butoxycarbonylamino)-4-phenyl-1-benzyl ketone (**I-18c**) was performed with $NaBH_4$ (**Scheme I-6**). The yield of the mixture of the two diastereomers was 47%, but poor diastereoselectivity was provided by the achiral agent. A 3:1 ratio of the two diastereomers **I-20 : I-21** resulted, as determined by ^1H NMR studies.

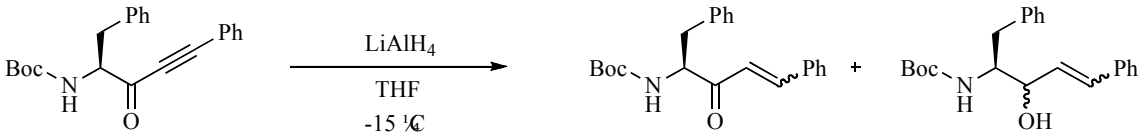
Scheme I-6



I-18 c **I-20** **I-21**

$LiAlH_4$ was also used in the reduction of **I-18c.** The reaction proceeded with chemoselective reduction of the triple bond to the alkene and partial reduction of the ketone (**I-18c**) (**Scheme I-7**), resulting in a complex mixture of the *cis* and *trans* isomers of both the ketone and allylic alcohol. The reaction was run using the same conditions as the reduction with $NaBH_4$, [16] but with a lower combined yield (31%).

Scheme I-7



I-18c **I-22** **I-23**

In an effort to improve diastereoselectivity, we performed the reduction of **I-18c** with diisobutylaluminum hydride (DIBAL-H) (**Figure I-9**) using a modified procedure reported by Martin.[17] As compared with $NaBH_4$, DIBAL-H gave us a similar yield (35%) and was less diastereoselective, as the ratio of propargyl alcohols **I-20 : I-21** was **1:1.5**.

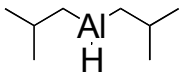
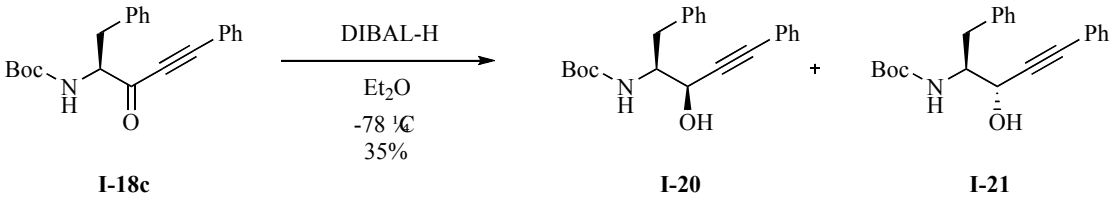


Figure I-9. DIBAL-H

Scheme I-8



I-18c **I-20** **I-21**

To summarize the effects of achiral reducing agents on the reduction of α-alkenyl ketone **I-18c**, all achiral reducing agents we used showed poor diastereoselectivity. In addition to the achiral reagents, we attempted to obtain diastereomerically pure 1,2-amino alcohol from **I-18c** by using chiral reducing agents. Based on the results reported in previous work,[18-21] our choice for a potentially successful chiral agent was the *R* and *S* enantiomers *B*-Isopinocampheyl-9-borabicyclo[3.3.1]nonane solution (Alpine Borane). The structure of the *R* enantiomer is shown in **Figure I-10**.

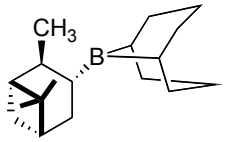
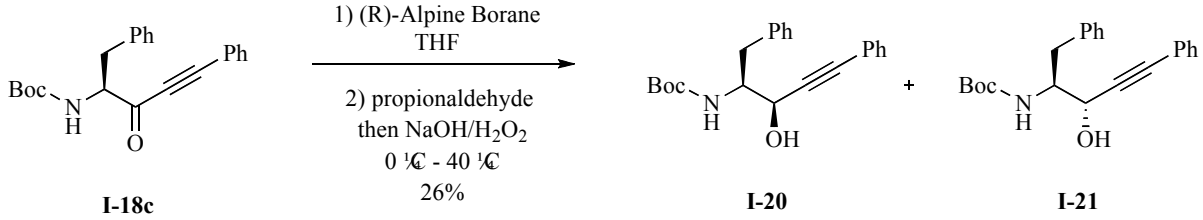


Figure I-10. *R*-Alpine Borane

Alpine Borane is a versatile chiral reagent capable of reducing a broad range of prochiral ketones with high enantiomeric purity. *R* and *S* Alpine Borane are especially useful reagents for the reduction of alkynyl ketones.[20,21] Reduction of **I-18c** with *R*-

Alpine Borane gave a combined yield of 26 % but with an excellent diastereoselectivity (>95:5 dr for the diastereomers **I-21 : I-20** as determined by ^{1}H-NMR.) (**Scheme I-9**)

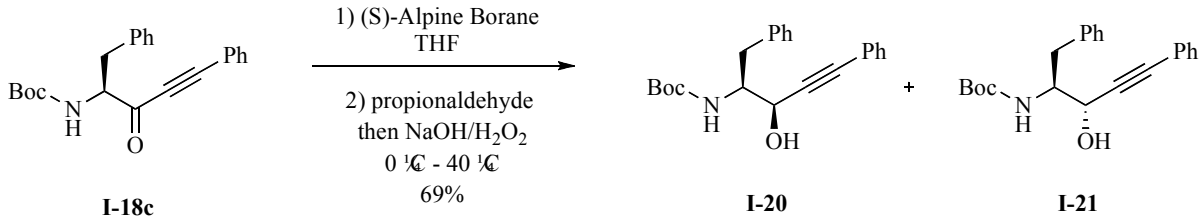
Scheme I-9



I-18c **I-20** **I-21**

Unfortunately, reduction of **I-18c** with *S*-Alpine Borane forms primarily the other diastereomer (**I-21**) with equal selectivity. Although we obtained a similar combined yield (29%), we also obtained a poor diastereomeric ratio of **I-21 : I-20** (**1:1.5**). The reduction with chiral agents *R*- and *S*-Alpine Borane was done according to modified published procedures[21,22] (**Schemes I-9** and **I-10**). In **Table I-2** the results obtained by treating compound **I-18c** with various reducing agents are summarized.

Scheme I-10



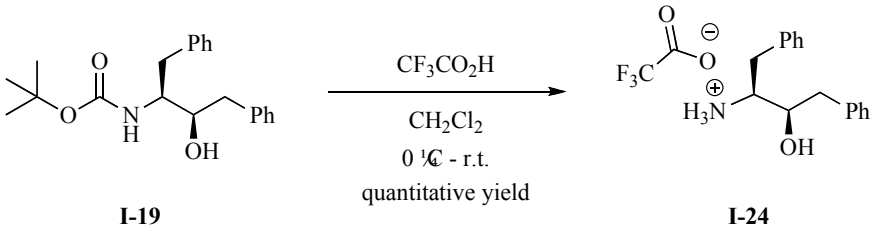
I-18c **I-20** **I-21**

Table I-3. Reduction of compound **I-18c** with various reducing agents.

Entry	Compound	Reducing agent	ratio		Yield (%)
			I-20	**I-21**	
1	**I-18c**	$NaBH_4$	3	1	47
2	**I-18c**	$LiAlH_4$	reduction of alkyne		--
3	**I-18c**	DIBAL-H	1	1.5	35
4	**I-18c**	*R*-Alpine Borane	>95	5	26
5	**I-18c**	*S*-Alpine Borane	1	1.5	69

The final step of our synthesis involved deprotection of the amino group in order to release the 1,2-amino alcohol (**Scheme I-11**).[22] Typical Boc-deprotection of amino alcohols requires acidic conditions. Trifluoroacetic acid was chosen as our deprotecting agent, and the final product (**I-24**) was isolated as the trifluoroacetate salt without further purification in a quantitative yield.

Scheme I-11



I-19 **I-24**

Conclusion

The five-step synthesis presented allows the preparation of a wide range of compounds containing a 1,2-amino alcohol moiety by using appropriate aliphatic and aromatic organometallic reagents in step 4. We started synthesis from L-phenylalanine, the natural occurring enantiomer, but our future work will involve also the use of the

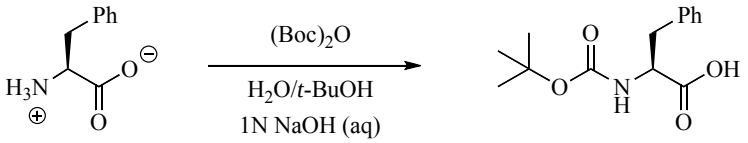
unnatural enantiomer, D-phenylalanine, and other amino acids. The building of the second chiral center in the amino acid structure, by reduction of the carbonyl group with achiral and chiral reducing agents, allows for preparation and isolation of each of the four diastereomers. Some of these diastereomers have not yet been synthesized, but work is ongoing in our laboratory. Additionally, improving diastereoselectivity for the formation of the *anti* diastereomer is a priority, as is unambiguously proving the stereochemistry of these compounds.

Experimental methods and data

I. General methods

All reactions were performed using commercially available anhydrous solvents, and used without further purification unless otherwise specified. Tetrahydrofuran was purified by distillation over sodium benzophenone ketal. Extraction and chromatography solvents were reagent grade and used without purification. All glassware utilized in the reactions was flame-dried or oven-dried before use. Thin layer chromatography (TLC) was performed on polyester-backed plates, coated with UV-active silica gel (250 μm thickness). A UV lamp and aqueous $KMnO_4$ solution were used for visualization of TLC plates. Purification of compounds by flash column chromatography was performed using silica gel (32-60 μm particle size, 60 Å pore size), and solvent as indicated. All ^{1}H and ^{13}C NMR data were obtained at room temperature on a 400 MHz JEOL ECX instrument, chemical shifts are expressed in δ (parts per million, ppm) and are reported relative to the solvent peak. Coupling constants (*J*) are expressed in Hertz. The splitting patterns are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Infrared spectra (IR) were recorded as thin films on NaCl plates using a Nicolet Impact 410 FTIR.

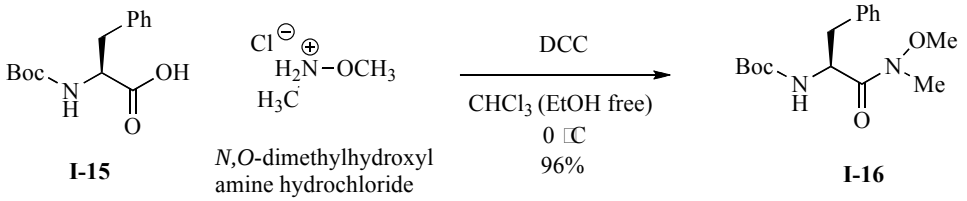
II. General procedure for N-BOC protection of amino acid (I-15)



L-Phenylalanine 96% **I-15**

L-phenylalanine (11.03 g, 63.4 mmol) was dissolved in 30 ml 1N NaOH solution. To this solution was added *tert*-butanol (8 ml), followed by the addition of di-*tert*-butyldicarbonate (1.742 g, 8.0 mmol). The pH of the solution was adjusted to about 12 by the addition of 1N NaOH as necessary. The mixture was stirred at room temperature overnight. The *tert*-butanol was removed *in vacuo*, and the aqueous residue was mixed with ethyl acetate and cooled to 0 ºC. The pH of the mixture was lowered to 2-3 by slow addition of a solution of 1M $KHSO_4$. The organic and aqueous phases were separated, and the aqueous residue was extracted with additional ethyl acetate (3 X 50 ml). The combined organic phase was washed with brine (3 X 50 ml), dried over Na_2SO_4, and concentrated *in vacuo* to afford 0.96 g (96%) of the *N*-protected amino acid (**I-15)** as a white powder. The product was used in the next step without further purification. ^1H NMR ($CDCl_3$, 400 Mhz) δ 7.30-7.25 (m, 5H), 4.92 (d, *J* = 8 Hz, 1H), 4.58 (m, 1H), 3.16 (dd, *J* = 4 Hz, 2H) 2.03 (s, 1H), 1.41 (s, 9H).

III. General procedure for synthesis of [(1S)-1-[(methoxymethylamino)carbonyl]-3-phenylethyl]-carbamic acid 1,1-dimethylethyl ester (**I-16**).

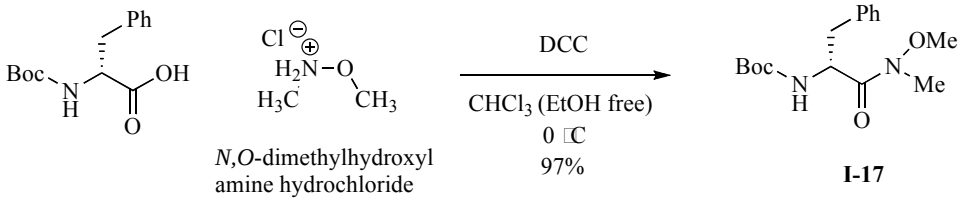


I-15 *N,O*-dimethylhydroxyl amine hydrochloride **I-16**

N-Boc-phenylalanine (5 g, 18.8 mmol) was mixed with *N,O'*-dimethylhydroxylamine hydrochloride (1.84 g, 18.8 mmol) in alcohol-free chloroform (38 ml) cooled with stirring to 0 °C in an ice bath. The chloroform was cooled in a refrigerator at least one hour before use. The cooled mixture was stirred for ten minutes, then *N*-methyl

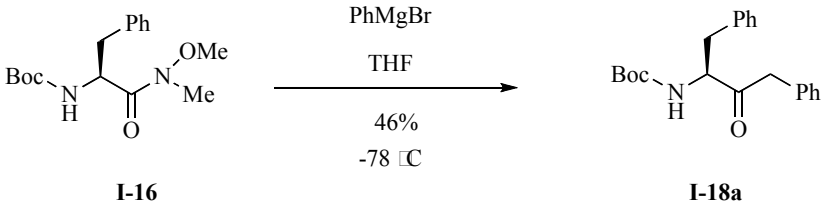
piperidine (2.28 ml, 18.8 mmol) was added. After the addition of piperidine, DCC (3.88 g, 18.8 mmol) was added portion-wise (about 0.5 g DCC per five minute interval), while the solution was stirred in the ice bath. The stirring was continued overnight, and the progress of the reaction was monitored by TLC. The solution was filtered and the residue was washed several times with cold chloroform. The filtrate was concentrated *in vacuo* and purified by flash column chromatography (30% ethyl acetate/hexane) to afford 4.80 g (96%) of **I-16**. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.25-7.15 (m, 5H), 5.13 (d, J = 8 Hz, 1H), 4.92 (m, 1H), 3.64 (s, 3H), 3.14 (s, 3H), 3.03 (dd, J = 4 Hz, 2H) 1.37 (s, 9H).

III A. [(1R)-1-[(methoxymethylamino)carbonyl]-3-phenylethyl]-carbamic acid 1,1-dimethylethyl ester (**I-17**).



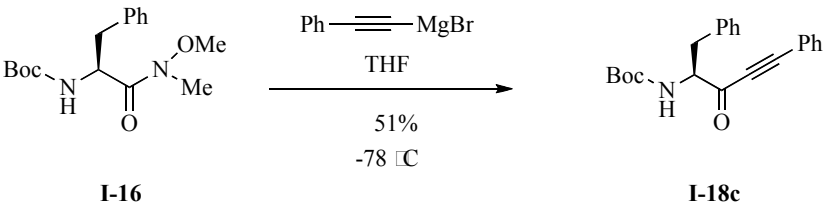
Obtained in 97% (3.215 g) from Boc protected amino acid **I-15** by the procedure **III** described above. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.24-7.16 (m, 5H), 5.13 (d, J = 8 Hz, 1H), 4.94 (m, 1H), 3.64 (s, 3H), 3.15 (s, 3H), 3.05 (dd, J = 12, 2H), 1.37 (s, 9H).

IV. **General procedure for addition of phenyl magnesium bromide to compound I-16 (I-18a).**



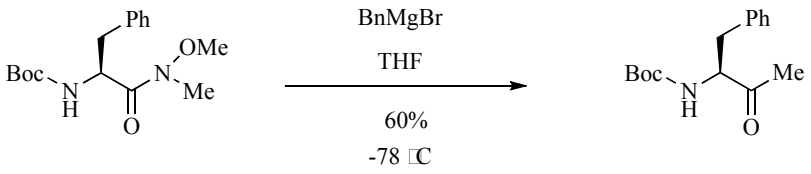
I-16 **I-18a**

Weinreb amide **I-16** (0.168 g, 0.541 mmol), synthesized using procedure **III** and stored in the refrigerator, was dissolved in THF (4 ml) and cooled to -78 ºC. To this solution, phenyl magnesium bromide (0.488 g, 2.38 mmol) was added dropwise and was stirred for 4 hours at -78 ºC. The reaction was quenched with 1N HCl (7 ml) and extracted with ethyl acetate (3 X 50 ml). The combined organic extract was washed with water (3 X 25 ml) and brine (3 X 25 ml), dried over Na_2SO_4, and concentrated *in vacuo*. The crude product was purified by flash column chromatography (10% ethyl acetate/hexane) to afford 0.220 g (46%) of **I-18a**. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.30-6.99 (m, 10H), 5.10 (d, 1H), 4.84 (m, 1H), 3.64 (q, *J* = 8 Hz, 2H), 3.01 (m, 2H), 1.41 (s, 9H).



I-16 **I-18c**

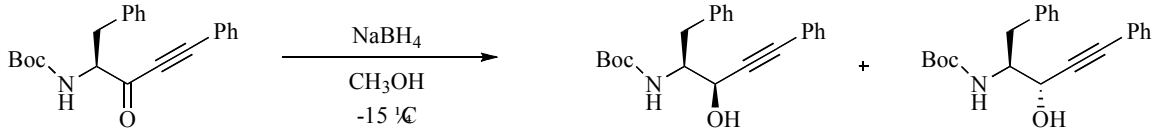
Alkynyl ketone **I-18c** was obtained in 51% yield (0.753 g) from Weinreb amide **I-15** and phenylethynylmagnesium bromide as described above in procedure **IV**. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.55-7.25 (m, 10H), 5.11 (d, *J* = 8 Hz, 1H), 4.78 (m, 1H), 3.27 (q, *J* = 8 Hz, 2H), 1.41 (s, 9H).



I-16 **I-18b**

Methyl ketone **I-18b** was obtained in 60 % yield from Weinreb amide **I-15** and methylmagnesium bromide as described above in procedure **IV**. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.42-7.33 (m, 10H), 5.66 (d, *J* = 8 Hz, 1H), 5.29 (m, 1H), 3.90 (m, 2H), 2.48 (s, 3H), 1.41 (s, 9H).

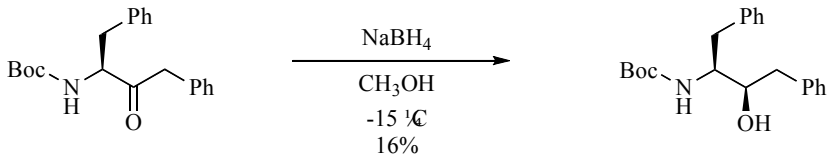
V. General procedure for reduction of α-amino ketone I-18c with $NaBH_4$ (I-20, I-21).



I-18 c **I-20** **I-21**

To a stirred solution of α-amino ketone **I-18c** (0.100 g, 0.28 mmol) in anhydrous methanol (0.60 ml) at -15ºC under a N_2 atmosphere was added $NaBH_4$ (0.032g, 0.84 mmol). After stirring for 30 minutes at –15 ºC, the mixture was gradually warmed to room temperature and quenched with a saturated aqueous solution of NH_4Cl (2 ml). The mixture was extracted with diethyl ether (3 X 25 ml). The combined organic phase was washed with 1N HCl (2 X 25 ml) and saturated $NaHCO_3$ (3 X 25 ml), dried over $MgSO_4$, and concentrated to give **I-20** and **I-21** in a combined yield of 0.047 g (47%) as a mixture of diastereomers. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.42-7.47 (m, 10H), 7.34-7.25 (m, 10H), 4.81 (d, *J* = 8 Hz, 1H), 4.63 (m, 1H), 4.18 (d, *J* = 8 Hz, 1H), 4.03 (m, 1H), 3.70 (m, 2H), 3.30 (s, 1H), 2.74 (m, 1H), 1.41 (s, 9H); 13C NMR (CD_3OD, 400 Mhz), δ 131.90,

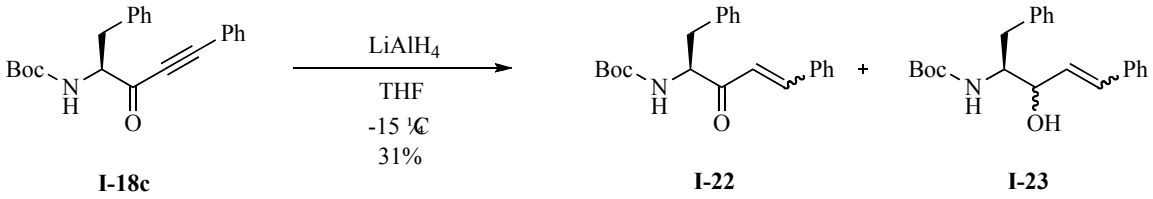
129.29, 128.72, 128.67, 128.47, 77.42, 77.09, 76.78, 65.68, 64.60, 57.02, 53.51, 37.77, 29.79, 28.37; IR (CH_2Cl_2) ν 3410, 3061, 3028, 2976, 2926, 2852, 1689, 1491, 1367, 1250, 1167, 1043, 1020, 1021, 757, 692.



I-18a **I-19**

Reduction of α-amino ketone I-18a with $NaBH_4$. Alcohol **I-19** was obtained in 16% yield from **I-18a** by the procedure **V** described above. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.25-7.20 (m, 10H), 4.99 (d, *J* = 4 Hz, 1H), 3.90 (m, 1H), 2.97 (dd, *J* = 4 Hz, 2H), 2.87 (m, 2H), 2.76 (m, 1H), 1.72, (s, 1H), 1.26, (s, 9H).

VI. General procedure for reduction of α-amino ketone I-18c with $LiAlH_4$ (I-22 and I-23).

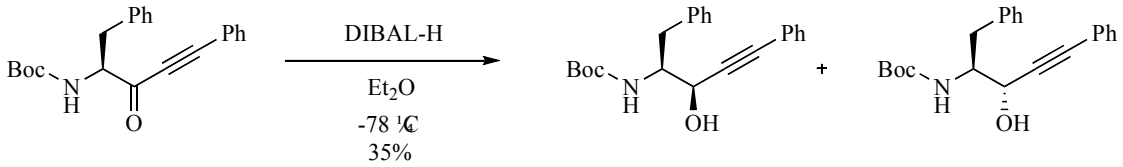


I-18c **I-22** **I-23**

To a stirred solution of α-amino ketone **I-18c** (0.080 g, 0.22 mmol) in anhydrous THF (2 ml) at –15 ºC under a N_2 atmosphere was added $LiAlH_4$ (0.01 g, 0.0027 mmol). After stirring for 30 minutes at –15 ºC, the mixture was gradually warmed to room temperature and quenched with a saturated aqueous solution of NH_4Cl (5 ml). The mixture was extracted with ethyl acetate (3 X 25 ml). The combined organic phase was washed with 1N HCl (2 X 25 ml) and saturated $NaHCO_3$ (3 X 25 ml), dried over $MgSO_4$, and concentrated to give the product mixture in a combined yield of 0.026 g (~31%). ^{1}H

NMR ($CDCl_3$, 400 Mhz), δ 7.34-7.25 (m, 10H), 6.64 (dd, *J* = 28 Hz, 1H), 6.23 (m, 1H), 4.82 (d, *J* = 8 Hz, 1H), 4.63 (m, 1H), 4.39 (d, 1H), 4.28 (m, 1H), 4.10 (m, 2H), 3.23 (d, *J* = 4 Hz, 1H), 2.97 (m, 1H), 2.89 (m, 2H), 2.44 (s, 1H), 1.35 (s, 9H), 0.85 (s, 9H).

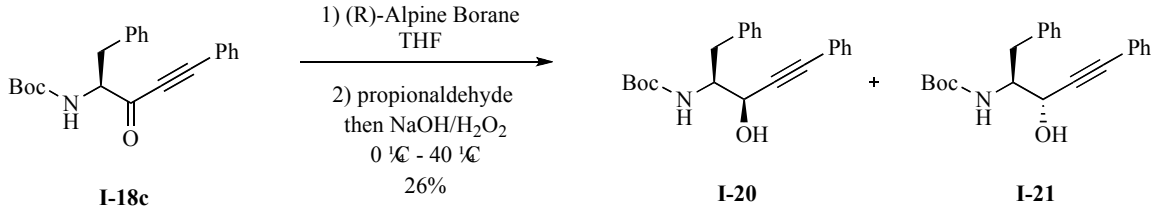
VII. General procedure for reduction of α-amino ketone I-18c with DIBAL-H (I-20 and I-21).



I-18c **I-20** **I-21**

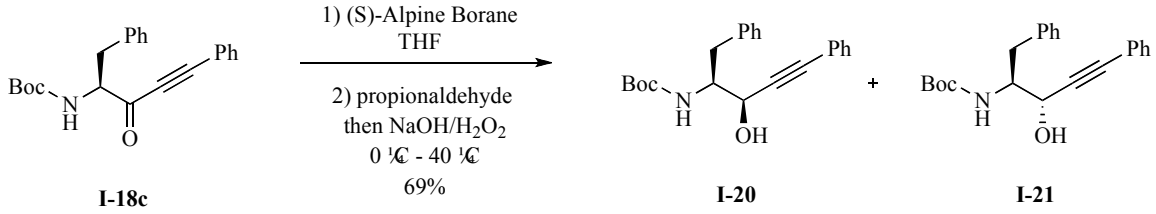
To a solution of **I-18c** (0.186 g, 0.53 mmol) in diethyl ether (2.5 ml), under N_2 at –78 ºC, DIBAL-H was added dropwise. The reaction mixture was allowed to warm to room temperature and stirred for an additional 2.5 hours. After 2.5 hours, the reaction was quenched with a solution of potassium sodium tartrate (4.5 ml) and stirred for another 3 hours. The mixture was extracted with ether (3 X 15 ml), washed with brine (3 X 20 ml), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting residue was purified by flash column chromatography (20% ethyl acetate/hexane) to afford a combined yield of 0.0646 g (35%) of the products **I-20** and **I-21**. ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.50-7.41 (m, 10H), 7.35-7.25 (m, 10 H), 4.58 (d, 1H), 4.63 (m, 1H), 4.16 (s, 1H), 4.04 (s, 1H), 3.71 (m, 2H), 3.49 (d, *J* = 8 Hz, 1H), 3.14 (d, 1H), 2.95 (m, 1H), 1.53 (s, 9H), 1.39(s, 9H).

VIII. General procedure for reduction of α-amino ketone I-18c with R-Alpine Borane I-20 and I-21).



I-18c **I-20** **I-21**

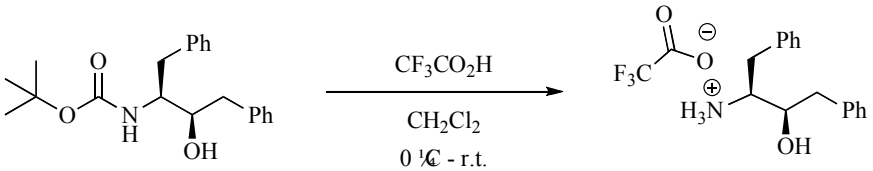
Ketone **I-18c** (0.350 g, 1.002 mmol) was dissolved at 0ºC in R-Alpine Borane (4 mmol, 0.5 M in THF). The mixture was stirred for 30 minutes at 0ºC, at which point the ice bath was removed and the mixture was stirred at room temperature overnight. Excess Alpine Borane was quenched by the addition of propionaldehyde (0.9 ml, 12.5 mmol) with external cooling (0 ºC), followed by stirring at room temperature for 1 hour. The solution was concentrated and α-pinene was removed under high vacuum at 40 ºC using a Kugelrohr distillation apparatus for 4 hours. The residue was diluted with THF (0.7 ml, 8.62 mmol), and solutions of NaOH (3M, 0.55 ml) and H_2O_2 (30%, 0.55 ml) were added dropwise at 0 ºC. The resulting mixture was stirred for 4 hours at 40 ºC, and then extracted with ether (3 X 30 ml). The combined organic phase was washed with brine (2 X 50 ml), dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The residue was purified by flash column chromatography (90% ethyl acetate / hexane) to afford **I-20** and **I-21** in a combined yield of 0.095g (26%). ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.30-7.26 (m, 10H), 4.86 (d, *J* = 8 Hz, 1H), 4.05 (m, 1H), 3.06 (dd, *J* = 16 Hz, 2H), 2.95 (d, *J* = 8 Hz, 1H), 2.83 (s, 1H), 1.38 (s, 9H).



I-18c **I-20** **I-21**

Reduction of α-amino ketone I-18c with S-Alpine-borane (I-20 and I-21). Obtained from α-amino ketone **I-18c** as described above in procedure **VI** to provide a combined yield of 0.017 g (69%). ^{1}H NMR ($CDCl_3$, 400 Mhz), δ 7.30 – 7.25 (m, 10H), 4.82 (d, *J* = 8 Hz, 1H), 4.58 (d, J = 16 Hz, 1H), 4.05 (m, 1H), 3.08 (dd, J = 16 Hz, 2H), 2.74 (s, 1H), 1.41 (s, 9H).

IX. Deprotection of compound α-amino ketone I-18a to form 1,2-amino alcohol (I-24).



I-19 **I-24**

To a 0 ºC solution of alcohol **I-18a** (0.040 g, 0.15 mmol) dissolved in MeOH (2 ml) was added CF_3CO_2H (6.14 g, 0.05 mmol). The reaction mixture was stirred at room temperature overnight. The reaction mixture was quenched with $NaHCO_3$ (10 ml) and diluted with CH_2Cl_2 (20 ml). The phases were separated and the aqueous phase was extracted with CH_2Cl_2 (3 X 10 ml). The combined organic phase was dried over $MgSO_4$, filtered, and concentrated under vacuum to afford **I-24** in quantitative yield (0.0433g). ^{1}H NMR (CD_3OD, 400 Mhz), δ 7.33-7.25 (m, 10H), 4.90 (s, 1H), 4.05 (m, 1H), 3.39 (m, 1H), 3.27 (dd, *J* = 4 Hz, 2H), 3.17 (dd, *J* = 12 Hz, 2H), 2.86 (m, 3H); 13C NMR

(CD_3OD, 400 Mhz), δ 129.13, 128.34, 128.01126.13, 125.84, 75.07, 56.77, 48.34, 48.13, 47.92, 47.70, 38.89.

Chapter II

Introduction

Plasminogen activator inhibitor-1 (PAI-1) is a serine protease inhibitor in the serpin family. Its main role is to regulate the plasminogen activation system, by inhibiting/regulating its activators tPA (tissue plasminogen activators) and uPA (urokinase plasminogen activators).[23] Activation of intravascular plasminogen by tPA and in migrating cells by uPA enables PAI-1 to inhibit not just intravascular fibrinolysis (the process of removing intravascular thrombi) but also proteolysis (the digestion of proteins by proteases) in the cells.[24] PAI-1 is released into circulatory system and extracellular matrixes under physiological conditions only by a few cells: liver cells, smooth muscle cells and platelets. Under pathological conditions, other cells such as tumor cells, endothelial cells, and other inflammation-activated cells release PAI-1.[24] PAI-1 interacts also with non-protease targets such as vitronectin, heparin, and many endocytic receptors of the low-density lipoprotein-receptors.[25]

Importance of proteases

Proteins that have served their purpose and are no longer needed must be degraded to their amino acid constituents, which are then recycled for the synthesis of new proteins. Breaking down of proteins to amino acids is accomplished by proteases. There are three main types of proteases: serine proteases, cysteine proteases, and aspartic proteases, which are defined by the amino acid that contains the nucleophilic functional group. In particular, serine proteases catalyze peptide bond cleavage in a two-stage process. The first is the nucleophilic attack of the serine hydroxyl on the peptide bond of the protein substrate. In this step, the N-terminus of the peptide chain is released and an

ester bond is formed between the active-site serine and the substrate. This covalent enzyme-substrate complex is called an acyl-enzyme intermediate. In the second step, the ester bond is hydrolyzed and the C-terminal is released.[26,27] This reaction is very slow; the half-life for the hydrolysis of a typical peptide bond, at a neutral pH, without catalyst, is estimated to be between 10 and 1000 years.[27] Yet peptide bonds must be hydrolyzed within milliseconds in some biological processes including protein degradation and the monitoring of active levels of other proteins.

Mechanism of inhibition of serine proteases by PAI-1

The structure of PAI-1 is characterized by high conformational plasticity that allows changes into many conformational states. Depending on the conditions, these conformational states are active, latent, protease-complex, or cleaved form.[28]

PAI-1 contains a reactive center loop (**Figure II-1a,** orange arrow) where the protease binds and cleaves the loop, forming the acyl-enzyme intermediate (protease-complex state). Prior to the hydrolysis of the acyl-enzyme intermediate, PAI-1 undergoes a massive change in conformation, trapping the target protease irreversibly in the acyl-enzyme state, thereby inhibiting its activity.[28] As a consequence of this structural shift, PAI-1 goes from the active state to an inactive state, in which it is unable to bind other proteases. Active, unbound PAI-1 can also spontaneously transform to an inactive latent conformation unless it is bound to the cofactor vitronectin, which stabilizes the active form of PAI-1.[29] Vitronectin is a glycoprotein that stabilizes the active form of PAI-1 by blocking its conformational shift, enabling the protease enzyme to release itself from PAI-1 after cleaving the reactive center loop.

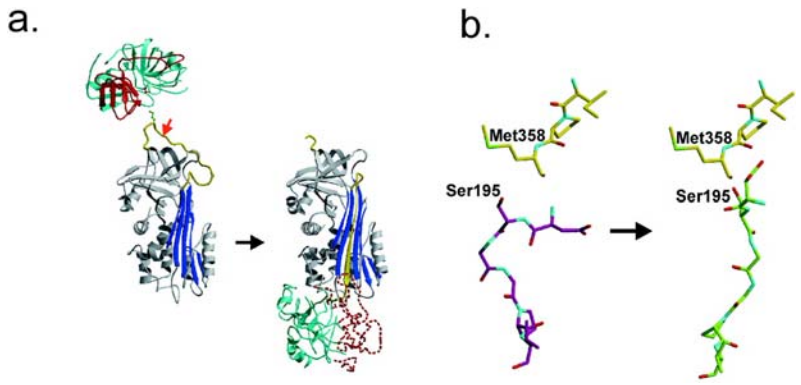


Figure II-1. Formation of the serpin-protease complex[26]

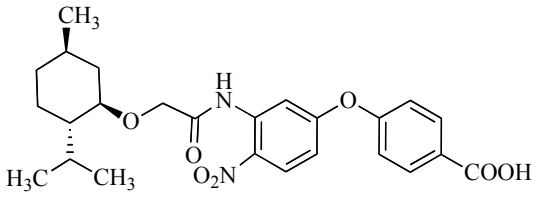
Normally, PAI-1 is present in low level in plasma, but chronic and acute diseases are associated with increased levels of PAI-1 expression and release. Acute diseases include sepsis and myocardial infarction, while chronic diseases include cancer,[30,31] type 2 diabetes,[32,33] and atherosclerosis. Morange *et al.*[34] and Linjen *et al.*[35] investigated the implication of high levels of PAI-1 in obesity. Studies conducted on mice revealed that genetically obese mice and diabetic mice deficient in PAI-1 had significantly reduced body weight and improved metabolism compared to obese mice with elevated PAI-1 in plasma. Other studies have shown that the PAI-1 secreted by tumor cells help to provide oxygen and nutrients for the growing tumor, leading to an increase of cell adhesion and migration.

Diabetes is a chronic disease that increases the risk of atherosclerosis. An increase in the levels of tPA (tissue plasminogen activator) and PAI-1 and a decrease in activity of tPA are indicators of increased risk of atherosclerosis.[33] *In vitro* studies demonstrated that C-reactive protein (CRP), which promotes atherosclerosis, also promotes PAI-1 expression.[32] It was found that in patients with diabetes and metabolic disorders, PAI-1

and CRP are both present in high levels, indicating a link between the root causes of atherosclerosis and diabetes.

Inhibitors of plasminogen activator inhibitor-1 (PAI-1)

Several small molecule compounds have been reported as PAI-1 inhibitors. Liang[35] and coworkers, in order to identify new inhibitors for PAI-1, screened a series of chemical compounds that showed activity against the protein. They found that several of these compounds contain a menthol core. The most potent compound was ZK4044 (**Figure II-2**) with an IC_{50} against PAI-1 of 644±255 μM and 100±90 μM in uPA- and tPA-based assays, respectively.

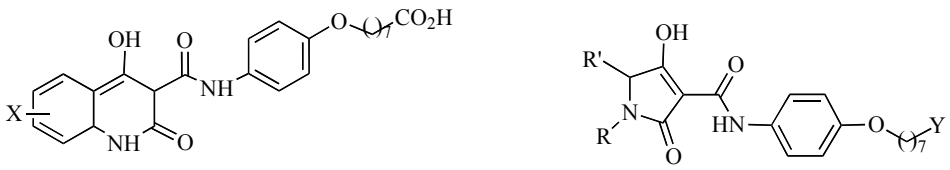


ZK4044

II-1

Figure II-2. The structure of ZK4044

Folkes[36] and coworkers synthesized a series of tetramic acid-based and hydroxyquinolinone-based inhibitors (**Figure II-3**) of plasminogen activator inhibitor (PAI-1).



Hydroxyquinoline based

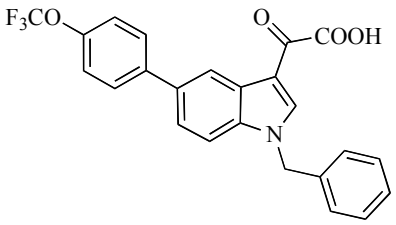
Tetramic acid based

II-2 **II-3**

Figure II-3. Inhibitors of PAI-1 synthesized by Folkes *et al.*[36]

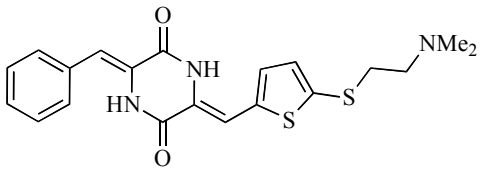
These templates allow the synthesis of a large variety of analogues by replacing R, R', Y, and X with different functional groups. It was found that the introduction of octanoic acid improved activity of both templates toward inhibition of PAI-1. Inhibition of PAI-1 was evaluated by measuring residual activity of tPA. The best results for tetramic acid-based inhibitors (IC_{50} = 0.69±0.06 μM) were obtained when the R' group was replaced with hydrogen, the R group with 4-Cl-Ph, and the Y group with a tetrazole moiety. In case of hydroquinolinone base, the best IC_{50} (0.50±0.14 μM) was obtained when X was replaced with 6-(benzo[b]thiophen-2-yl) and Y with CO_2H.

Elkodah[23] and coworkers used high-throughput screening to identify new inhibitors of PAI-1. Using scaffolds of the confirmed hits, they found multiple classes of compounds that all had in common a carboxylic acid or an acid bioisostere. Among these compounds, they found the indole oxoacetic acid scaffold to be the most successful structure explored, which led them to the discovery of tiplaxtinin (**Figure II-4**).



Tiplaxtinin
IC_{50} = 2.5 μM

II-4

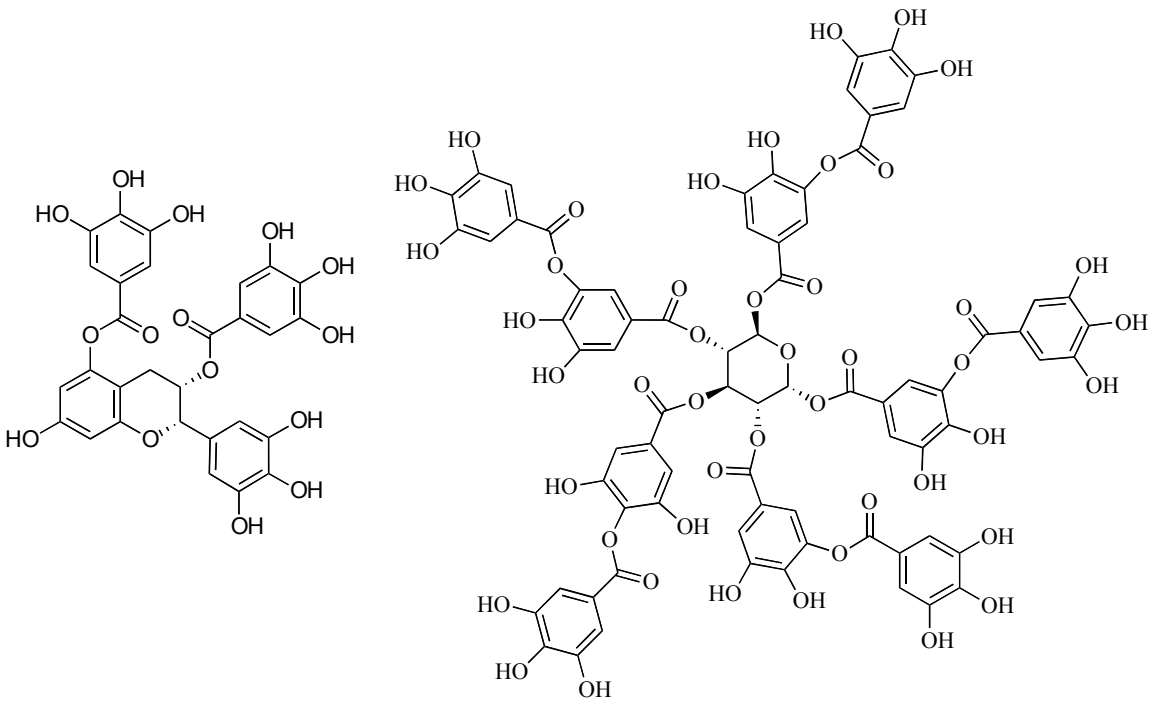


XR5118
IC_{50} = 8 μM

II-5

Figure II-4. Inhibitors of PAI-1 synthesized by Elkodah *et al.*[23]

Daniel Lawrence and coworkers at the University of Michigan Medical School screened a library of 3,000 diverse compounds for activity against PAI-1 (unpublished data). Compounds inhibiting PAI-1 activity by more than three standard deviations greater than the negative controls were selected for further analysis. In the majority of the hits, two classes of scaffold units were observed: gallate-containing carbohydrates and catechins. Two of the compounds identified, tannic acid and epigallocatechin-3,5-digallate (**Figure II-5**), showed the highest activity against PAI-1. Both compounds have in common multiple galloyl units attached to a carbohydrate in the case of tannic acid and a catechin in the case of epigallocatechin-3,5-digallate.



Epigallocatechin-3,5-digallate
IC_{50} = 7.29 μM

Tannic acid
IC_{50} = 2.54 μM

II-6 **II-7**

Figure II-5. Inhibitors of PAI-1 discovered by Lawrence *et al.*[37]

The aim of the project described here is to identify new inhibitors with structures based on these gallate-containing compounds, and test them for activity against PAI-1. Dr. Daniel Lawrence and Dr. Mark Warnock performed biological screens of all compounds at the University of Michigan.

Based on the structure of the two active compounds discovered from the library screen, tannic acid and epigallocatechin-3,5-digallate, there were two questions that we wanted to answer: 1) is the structure of the central core important, and 2) do the number and position of galloyl units around the central scaffold influence the activity of the inhibitors against PAI-1? Our first approach was to attach multiple galloyl units to

various carbohydrate molecules and test the resulting compounds against the PAI-1. The second approach was to attach galloyl units to both ends of tethers of various lengths.

Results and Discussion

Introduction

The aim of the work described here is the chemical synthesis of compounds with various carbohydrate or dihydroxy cores that are attached to multiple galloyl units, and the biological testing of these compounds against the plasminogen activator inhibitor-1 (PAI-1). In order to establish a structure-activity relationship (SAR) between our inhibitors and PAI-1, we intend to answer the following question: is the identity of the central core or the number of galloyl groups attached to the core important factors in determining activity against PAI-1. In order to further probe the structure-activity relationship, we have also synthesized, in the second portion of this work, a series of tethered compounds with linkers of various lengths that have attached at each end a galloyl unit. Based on studies by Lawrence *et al*,[37] compounds of this class are possible inhibitors of PAI-1.

The first step towards developing a structure-activity relationship (SAR) was to vary the stereochemistry around the central core by using different carbohydrates. We started our synthesis with three sugars: D-glucopyranose, D-galactopyranose, and D-mannopyranose (**Figure II-6**).

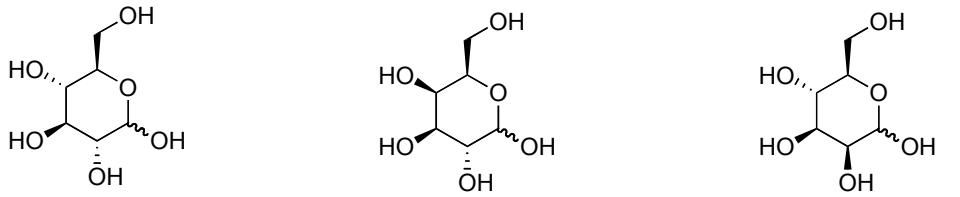
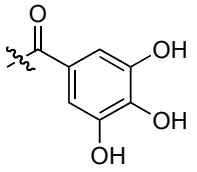


Figure II-6. Core carbohydrates used in synthesis

The second step in building our SAR was to attach multiple galloyl units (**Figure II-7**) to each of the five hydroxyl groups of the carbohydrate, followed by *in vitro* biological testing in order to determine if any of the carbohydrates proved a more potent inhibitor than tannic acid, and to determine the effect of the carbohydrate core stereochemistry on PAI-1 inhibition.



II-11

Figure II-7. Structure of galloyl group ('**G**')

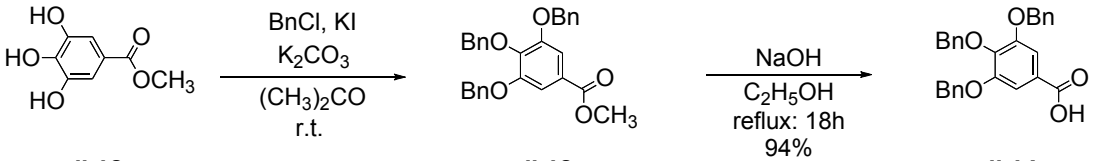
The synthetic route for preparation of the pentagalloyl carbohydrates was modified from that reported by Ren *et al.*[38] The first step of the three-step synthesis was alkylation of the three-hydroxyl groups of methyl gallate with benzyl chloride, followed by basic hydrolysis of the benzyl-protected ester to give the benzyl-protected gallic acid. This acid was used in the next step, a Steglich esterification. In this step, the acid is esterified with the hydroxyl groups of the carbohydrate. The last step of our synthesis was the hydrogenolysis of the benzyl protecting groups, releasing the desired pentagalloyl carbohydrate.

Synthesis of pentagalloyl carbohydrates

The first step of our synthesis was an alkylation reaction, in which methyl gallate (**II-12**) was allowed to react with benzyl chloride and catalytic potassium iodide to form methyl-3,4,5-tribenzyloxybenzoate (**II-13**) (**Scheme II-1**). The reaction proceeds via an

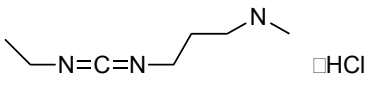
S_N2 mechanism, where the active intermediate is benzyl iodide, produced by a halogen exchange reaction between KI and benzyl chloride. In the second step, basic hydrolysis of methyl-3,4,5-tribenzyloxybenzoate **II-13** with NaOH forms the sodium salt, which is protonated upon acidification to form 3,4,5-tribenzyloxybenzoate (**II-14**) in 94% yield over two steps.

Scheme II-1

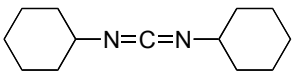


II-12 **II-13** **II-14**

The next step, a Steglich esterification,[39] is a coupling reaction between the hydroxyl groups of a carbohydrate and the carboxylic group of compound **II-14** using N,N'-dicyclohexylcarbodiimide (DCC) and N,N'-(dimethylamino)pyridine (DMAP) as the coupling agents. One of the disadvantages of using DCC (**Figure II-8**) as a coupling agent is that the byproduct, N,N'-dicyclohexylurea (DCU), is difficult to remove. To overcome this problem, the reaction was performed using 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride (EDC) (**Figure II-8**), a water soluble coupling agent. EDC is also soluble in many organic solvents, like CH_2Cl_2, THF, and DMF.[40] The byproduct formed using this coupling agent, 1-ethyl-3-(3-dimethylaminopropyl) urea, is removed more easily by extraction with dilute acid.



1-ethyl-3-(3-dimethylaminopropyl) carbodiimide hydrochloride



N,N'-dicyclohexylcarbodiimide

Figure II-8. Coupling agents used in the Steglich esterification

The esterification of D-glucopyranose using EDC (**Scheme II-2**) gave a yield of 9.5%, while esterification using DCC (**Scheme II-3**), gave a quantitative yield due to the presence of the byproduct, DCU, which could not be removed entirely from the reaction. Due to the size of the molecules in the following figures, the benzyl group is represented by 'Bn' (**Figure II-9**).

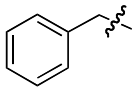
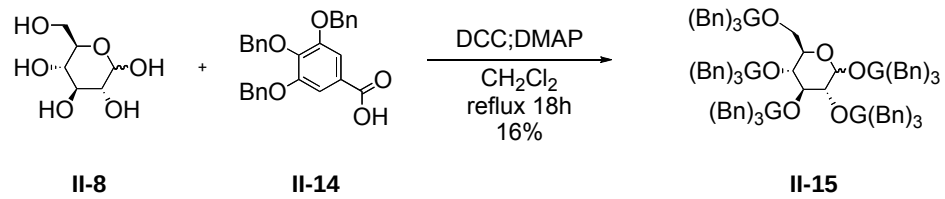


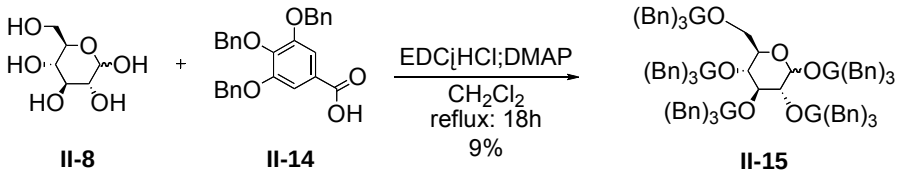
Figure II-9. Benzyl group ('**Bn**')

Scheme II-2



II-8 **II-14** **II-15**

Scheme II-3

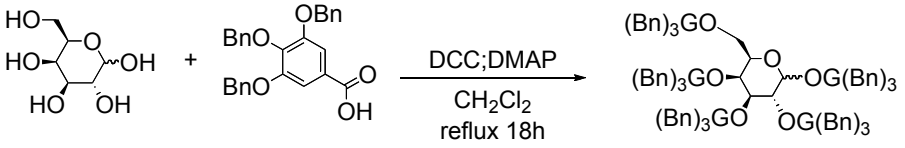


II-8 **II-14** **II-15**

The esterification of D-galactopyranose (**Scheme II-4**) and D-mannopyranose (**Scheme II-5**) with compound **II-14** were performed using DCC as a coupling agent, and with similar conditions as the esterification of D-glucopyranose. The pentaesterified α-D-galactopyranose (**II-16**) was isolated in 13% yield, the β-D-galactopyranose (**II-17**) in

31% yield, and the D-mannopyranose (**II-18**) in 18% yield. The separation of both α and β anomers by flash column chromatography was performed only for D-galactopyranose. Only the β anomer of the D-mannopyranose was isolated, while for the D-glucopyranose separation was not attempted, and a mixture of both anomers, α and β, was carried forward.

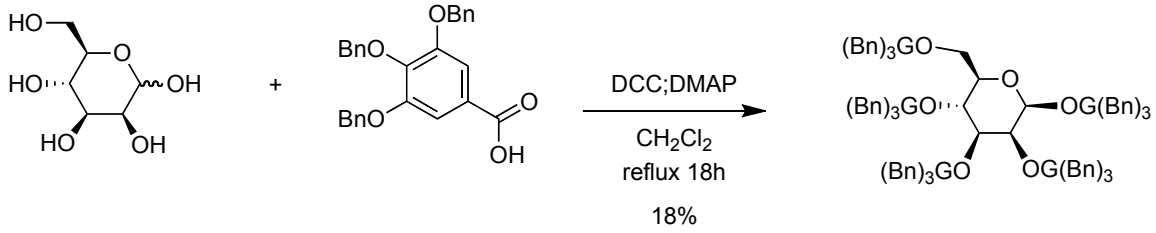
Scheme II-4



II-9 **II-14**

II-16: α, 13%
II-17: β, 31%

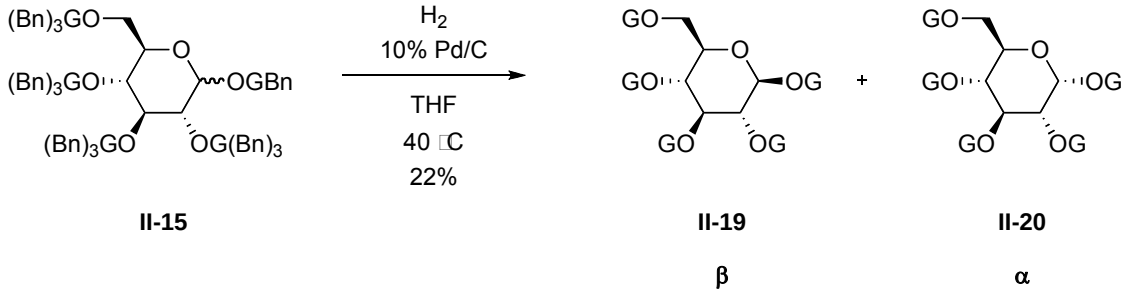
Scheme II-5



II-10 **II-14** **II-18**

Hydrogenolysis is a reaction where a benzylic carbon-heteroatom single bond is cleaved by hydrogen. The purpose of this step was to remove the benzyl protecting groups and form the final product, pentagalloyl carbohydrate. The hydrogenolysis reaction was performed using 10% palladium on carbon as a catalyst, and tetrahydrofuran as a solvent.[38]

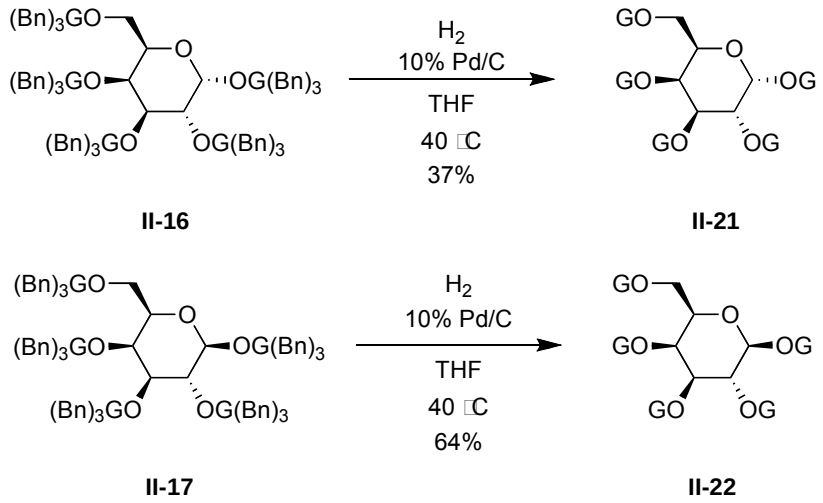
Scheme II-6



The hydrogenolysis of D-glucopyranose-based **II-15** (**Scheme II-6**), after purification by flash column chromatography, provided a mixture of α and β anomers. By comparison of the NMR with similar published compounds,[41] the β anomer was identified as the major component of the mixture, contained in an α : β ratio of 1 : 4.

The hydrogenolysis of compounds **II-16** and **II-17** with D-galactopyranose core is showed in **Scheme II-7.**

Scheme II-7

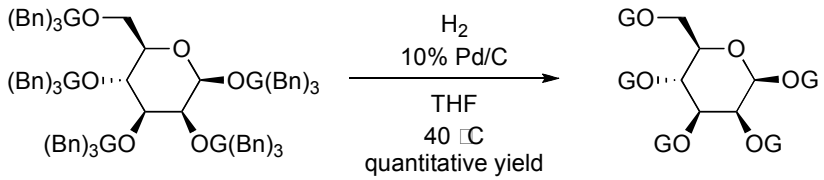


Hydrogenolysis performed on compound **II-16** provided the α-anomer in a 37% yield. Hydrogenolysis of **II-17**, provided the β-anomer as the major product, while a byproduct carried from the previous step, possibly a furanose version of the compound,

was also present in a 2 : 1 ratio of β-anomer to byproduct. The reaction proceeded with a combined yield of 64%.

The hydrogenolysis performed on compound **II-18 (Scheme II-8)**, led us to the fully deprotected β-anomer in quantitative yield. By ^{1}H-NMR analysis, the purity of β-anomer is higher than 95%.

Scheme II-8



II-18 **II-23**

Biological testing against plasminogen activator inhibitor-1 (PAI-1) of these compounds was performed at the University of Michigan Medical School by Daniel Lawrence and Mark Warnock. The compounds sent for testing were the pentagallate esters (**II-19** through **II-23**) and the fully benzylated α- and β-anomers of D-galactopyranose (**II-16** and **II-17**). The activity of the inhibitors against PAI-1 was measured by the determination of each compound's IC_{50}, which represents the concentration of the inhibitor required for 50% inhibition of its target (PAI-1). Hence, lower IC_{50} values indicate more potent inhibitors. The results of biological testing are summarized in **Table II-1**.

Table II-1. IC_{50} of inhibitors with various carbohydrates cores.

Entry	Compound	Core	α / β	IC_{50} (μM)
1	**II-16**	galactose	α	>200
2	**II-17**	galactose	β	>200
3	**II-19 + II-20**	glucose	α + β	76
4	**II-21**	galactose	α	52
5	**II-22**	galactose	β	68
6	**II-23**	mannose	β	59

From the results summarized in **Table II-1** we can conclude that the best inhibitor of PAI-1 from this group was compound **II-21**, the deprotected pentagallate ester with the α-galactose core. However, the small difference between IC_{50} values of compounds with various carbohydrate cores demonstrates that the stereochemistry of the carbohydrate core does not seem to play a crucial role in PAI-1 inhibition. In addition, the lack of inhibition by the benzylated compounds **II-16** and **II-17** at the range of concentrations tested indicates that the free hydroxyl groups are required for potency. Our next approach was to probe if the number and spacing of the galloyl groups around the central core influence inhibition of PAI-1.

Synthesis of digalloyl-based inhibitors

In the second phase of this project, we synthesized and tested digalloyl esters connected with hydrocarbon linkers of various lengths against PAI-1. The synthetic route used for formation of these diesters was the same as used for the synthesis of the

pentagalloyl carbohydrates. The four diol linkers used as cores are shown in **Figure II-10**.

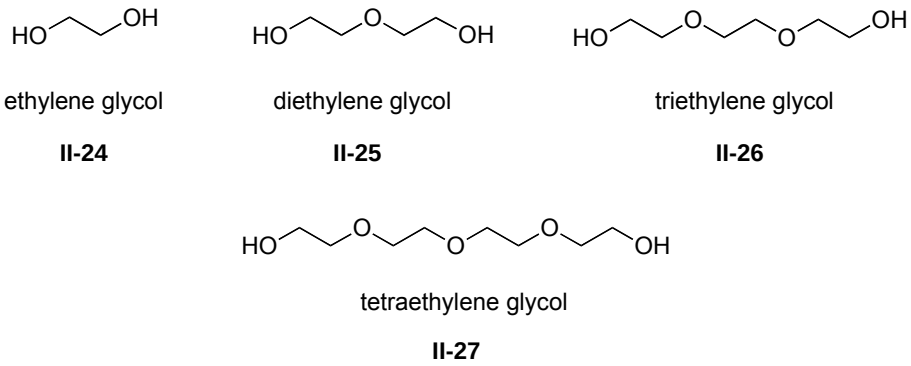


Figure II-10. Diols used as linkers/tethers

We started our synthesis with the esterification of the hydroxyl groups of diols **II-24, II-25, II-26** and **II-27** with 3,4,5-tribenzyloxybenzoate (**II-14**). The results of these reactions are summarized in **Scheme II-9** and **Table II-2**:

Scheme II-9

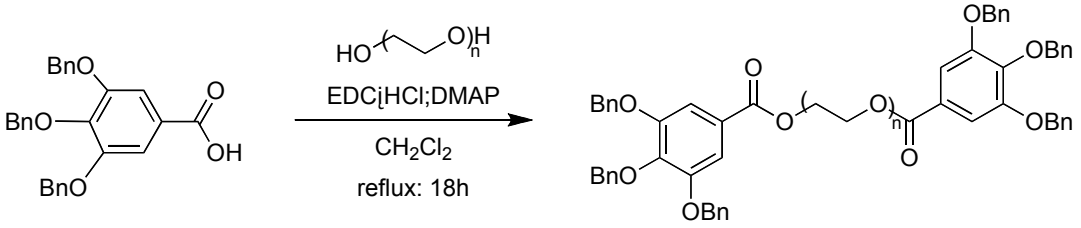
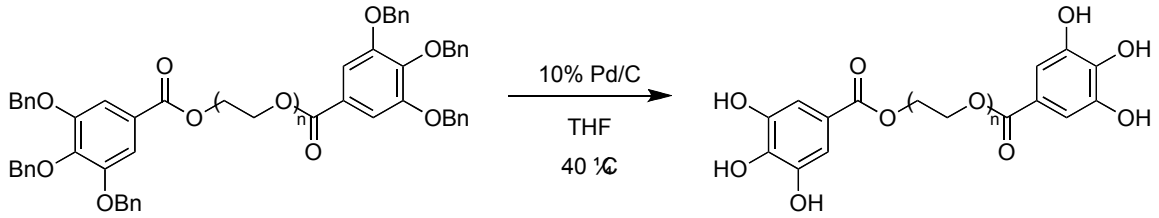


Table II-2. Steglich esterification of mono-, di-, tri- and tetra-ethylene glycol with **II-14**

Entry	Compound #	n	Yield %
1	**II-28**	1	14
2	**II-29**	2	30
3	**II-30**	3	27
4	**II-31**	4	11

In the next step (**Scheme II-10**), the benzyl-protecting group was removed by hydrogenolysis using palladium on carbon as a catalyst. **Table II-3** summarizes the results of the hydrogenolysis reaction.

Scheme II-10



II-28: n = 1 **II-30**: n = 3
II-29: n = 2 **II-31**: n = 4

II-32: n = 1 **II-34**: n = 3
II-33: n = 2 **II-35**: n = 4

Table II-3. Hydrogenolysis of digalloyl compounds

Entry	Compound #	n	Yield %
1	**II-32**	1	quantitative
2	**II-33**	2	44
3	**II-34**	3	51
4	**II-35**	4	14

Biological testing against PAI-1 was again performed by Dr. Daniel Lawrence and Mark Warnock at the University of Michigan Medical School. The results are shown in **Table II-4**.

Table II-4. IC_{50} values of digalloyl inhibitors with tethered core

Entry	Compound	# of glycol units	IC_{50} (μM)
1	**II-32**	1	0.3
2	**II-33**	2	180
3	**II-34**	3	1300
4	**II-35**	4	1714

Among the inhibitors synthesized, compound **II-32**, 1,2-bis-galloyloxyethane, with an IC_{50} of 0.3 μM, showed a surprising level of activity against PAI-1. To our knowledge, this level of potency makes it a full order of magnitude more effective versus PAI-1 than any other inhibitor, including tannic acid (IC_{50}: 2.54 μM), tiplaxtinin (IC_{50}: 2.5 μM), and epigallocatechin-3,5-digallate (IC_{50}: 7.29 μM). In addition, a clear pattern of activity emerged, showing that as the distance between the galloyl groups increased, potency rapidly decreased by several orders of magnitude. A more specific determination of the optimal distance between the galloyl groups is necessary.

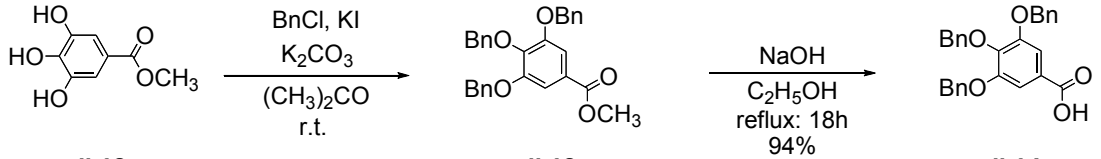
These promising early results make us optimistic that with further investigation we will be able to obtain even more potent inhibitors of PAI-1. Future work in the Emal laboratory will include the synthesis of compounds with more rigid linkers, determination of which hydroxyl groups of the galloyl groups are essential for activity, and an exploration of the pH-dependence of inhibitor activity.

Experimental Methods and Data

I. General methods

All procedures were carried out using commercially available anhydrous solvents and were used without further purification, unless noted otherwise. THF was purified by distillation over sodium benzophenone ketal. All extraction and chromatography solvents were reagent grade and used without purification. Thin layer chromatography (TLC) was performed on polyester-backed plates coated with UV-active silica gel (250 μM thickness). A UV lamp and aqueous $KMnO_4$ solution were used for visualization of TLC plates. Purification of compounds using flash column chromatography was performed using silica gel (32-60 μm particle size, 60 Å pore size) and solvent as indicated. All ^{1}H and ^{13}C NMR data were obtained on a 400 MHz JEOL ECX instrument, chemical shifts are expressed in δ (parts per million, ppm), and coupling constants (*J*) are expressed in hertz (Hz). The splitting patterns are abbreviated as follows: s (singlet), d (doublet), t (triplet), q (quartet), dd (doublet of doublets), and m (multiplet). Infrared (IR) spectra were recorded as thin films on a NaCl plate using a Nicolet Impact 410 FTIR.

II. General procedure for synthesis of 3,4,5-tribenzylbenzoate (II-4)



II-12 **II-13** **II-14**

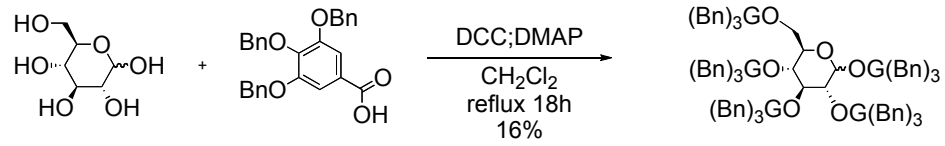
A mixture of methyl-3,4,5-trihydroxybenzoate (10 g, 54.3 mmol), potassium iodide (4 g, 27.7 mmol), and anhydrous K_2CO_3 (44 g, 318 mmol) in high-purity grade acetone (500 ml) was stirred at room temperature for 20 minutes. Benzyl chloride (20 ml, 173.8 mmol) in acetone (100 ml, 1.360 X 10^3 mmol) was added to the solution. The suspension was

refluxed for 20 hours under N_2 atmosphere. TLC plates showed full conversion of the methyl-3,4,5-trihydroxybenzoate to carboxylic acid **II-14.** The solid was filtered off and the filtrate evaporated. The residue was taken up in 400 ml dichloromethane. The suspension was vacuum filtered through Celite, and the filtrate was evaporated and dried under vacuum for 3 hours. Methyl-3,4,5-tribenzyloxybenzoate was obtained as a white solid and used without further purification.

Crude methyl-3,4,5-tribenzyloxybenzoate (22.54 g) was suspended in 500 ml of 95% ethanol, and 3.54 g (88.5 mmol) of sodium hydroxide was added. The mixture was refluxed under N_2 atmosphere for 2 hours. After 2 hours, the hot solution was poured into 525 ml of 0.6 M hydrochloric acid, stirred for 10 min., and the solid was filtered off. The crude product was washed successively with 1:1 95% ethanol and water (100 ml), water (100 ml), 95% ethanol (100 ml), methanol (2 x 50 ml), and tert-butyl methyl ether (2 x 50 ml). The solid was dried overnight under vacuum and afforded 21.19 g (94%) of **II-14**. ^{1}H NMR ($CDCl_3$, 400 MHz δ 7.42-7.25 (m, 17H), 5.05 (m, 6H), 3.48 (s, 4H), 2.58 (s, 1H).

III. General procedure for coupling reaction (Steglich esterification)

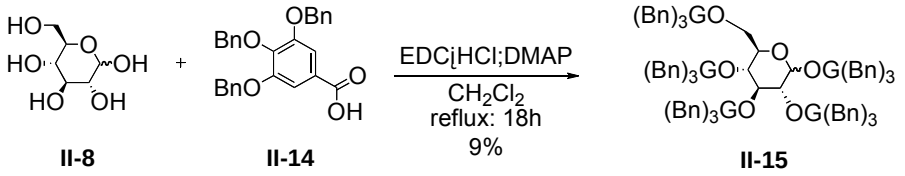
III A. D-Glucopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] (DCC) (II-15)



II-8 **II-14** **II-15**

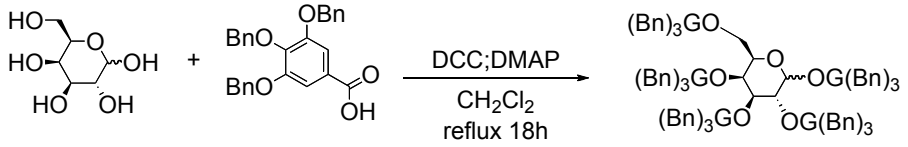
A mixture of glucose (0.21 g, 1.17 mmol), 3,4,5-tribenzyloxybenzoate (3.77 g, 8.57 mmol), DCC (2.2 g 10.68 mmol), and *N*,*N*-dimethylaminopyridine (1.2 g, 9.84 mmol) in dichloromethane (135 ml) was refluxed for 20 hours. The mixture was cooled to room temperature, filtered, and the filtrate was evaporated. The crude compound was purified by flash column chromatography (75 : 24 : 1 CH_2Cl_2: toluene : ethyl acetate) and afforded a 16% yield of 0.446 g of **II-15**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45–7.15 (m, 85 H), 6.21 (d, *J* = 8.0 Hz, 1H), 6.05 (t, *J* = 10.0 Hz, 1H), 5.84 (dd, *J* = 10.0, 8.0 Hz, 1H), 5.73 (t, *J* = 10.0 Hz, 1H), 5.14-4.92 (m, 30H), 4.76 (m, 1H), 4.44 (m, 1H), 4.35 (m, 1H).

III B. D-Glucopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] (EDC) (II-15)



A mixture of glucose (**II-8**) (0.21 g, 1.17 mmol), 3,4,5-tribenzyloxybenzoate (3.77 g, 8.57 mmol), EDC (2.04 g, 10.68 mmol), and *N*,*N*-dimethylaminopyridine (DMAP) (1.2 g, 9.84 mmol) in dichloromethane (135 ml) was refluxed for 18 hours. The mixture was cooled to room temperature while stirring. The organic layer was washed with 10% citric acid (3 X 50 ml), saturated $NaHCO_3$ (2 X 50 ml) and brine (2 X 30 ml). The combined organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The crude compound was purified by flash column chromatography (75 : 24 : 1 CH_2Cl_2: toluene : ethyl acetate), affording 0.253 g (9%) of **II-15**. ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45–7.15 (m, 85H), 6.21 (d, *J* = 8.0 Hz, 1H), 6.05 (t, *J* = 10.0 Hz, 1H), 5.84 (dd, *J* =

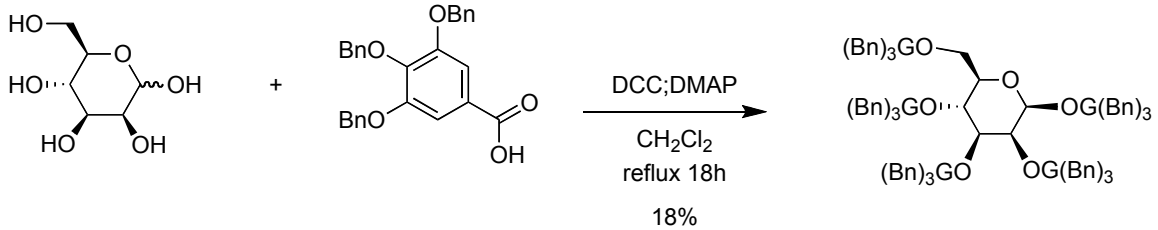
10.0, 8.0 Hz, 1H), 5.73 (t, *J* = 10.0 Hz, 1H), 5.14-4.92 (m, 30H), 4.76 (m, 1H), 4.44 (m, 1H), 4.35 (m, 1H).



II-9 **II-14** **II-16**: α, 13%
II-17: β, 31%

α-D-galactopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] II-16. Obtained in 13% yield from galactose (**II-9**) and 3,4,5-tribenzyloxybenzoate (**II-14**) by the same procedure as for compound **II-15.** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.42-7.27 (m, 85H), 6.11 (dd, J = 16 Hz 1H), 6.02 (d, 1H) 5.08-4.80 (m, 30H), 4.65 (m, 2H), 4.47 (d, *J* = 8 Hz, 1H), 4.14 (m, *J* = 20, 1H), 3.70 (m, 1H), 3.47 (m, 1H).

β-D-galactopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] II-17. Obtained in 31% yield from galactose (**II-9**) and 3,4,5-tribenzyloxybenzoate (**II-14**) by the procedure **III A.** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.45-7.15 (m, 85H), 6.20 (d, J = 8, 1H), 6.11 (d, J = 12, 1H), 6.04 (d, J = 4, 1H), 5.81 (m, 2H), 5.12-4.94 (m, 30 H), 4.47 (t, J = 8 Hz, 1H), 4.13 (m, 1H), 3.71 (m, 1H).

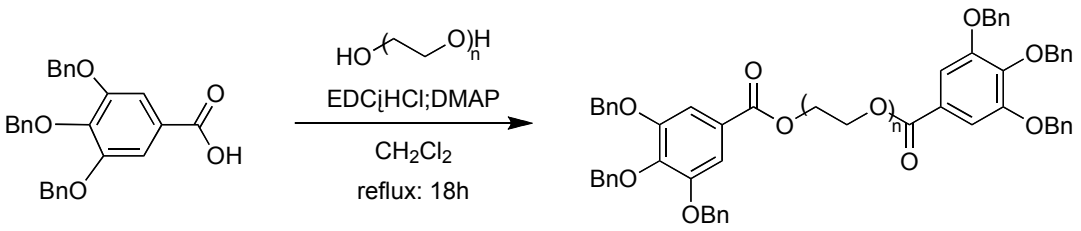


II-10 **II-14** **II-18**

α-D-mannopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] II-18. Obtained in 18% yield from mannose (**II-10**) and 3,4,5-tribenzyloxybenzoate (**II-14**) by the procedure **III A.** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.28-7.17 (m, 85H), 6.56 (d, *J* = 2 Hz, 1H), 6.20 (t,

J = 20 Hz, 1H), 6.03 (dd, *J* = 12 Hz, 2H), 5.86 (t, *J* = 4 Hz, 1H), 5.00-4.84 (m, 30H), 4.57 (m, 1H), 3.71 (m, 1H), 3.47 (m, 1H).

IV. General procedure for the formation of digalloyl compounds



II-14

II-28: n = 1 **II-30**: n = 3
II-29: n = 2 **II-31**: n = 4

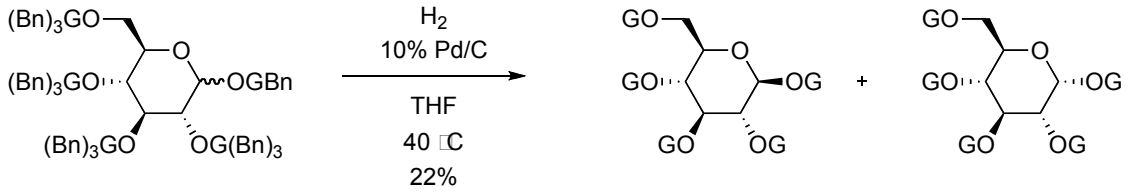
Di-*O*-(3,4,5-tribenzyloxybenzoyl)ethylene glycol II-28. A mixture of ethylene glycol (**II-24**) (0.124 g, 2 mmol), 3,4,5-tribenzyloxybenzoate (**II-14**) (2.60 g, 6 mmol), EDC (1.43 g, 10.68 mmol), and *N*,*N*-dimethylaminopyridine (DMAP) (0.84 g, 6.9 mmol) in dichloromethane (120 ml) was refluxed for 20 hours. The mixture was cooled to room temperature while stirring. The organic layer was washed with 10% citric acid (3 X 50 ml), saturated $NaHCO_3$ (2 X 50 ml), and brine (2 X 30 ml). The organic phase was dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum. The crude compound was purified by flash column chromatography (90% ethyl acetate/hexane), to afford 0.258 g (14%) of **II-28**.^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.40–7.20 (m, 34H), 5.04 (m, 12H), 4.59 (s, 4H).

Di-*O*-(3,4,5-tribenzyloxybenzoyl)diethylene glycol II-29. Obtained in 30% yield from diethylene glycol (**II-25**) and 3,4,5-tribenzyloxybenzoate (**II-14**) by the procedure described above for compound **II-28.** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.37–7.25 (m, 34H), 5.05 (m, 12H), 4.45 (m, 4H), 3.84 (m, 4H).

Di-*O*-(3,4,5-tribenzyloxybenzoyl)triethylene glycol II-30. Obtained in 27% yield from triethylene glycol (**II-26**) and 3,4,5-tribenzyloxybenzoate (**II-14**) by the procedure described above for compound **II-28.** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.56–7.25 (m, 34H), 5.09 (m, 12H), 4.41 (m, 4H), 3.79 (4H), 3.68 (s, 4H).

Di-*O*-(3,4,5-tribenzyloxybenzoyl)tetraethylene glycol II-31. Obtained in 11% yield from tetraethylene glycol (**II-27**) and 3,4,5-tribenzyloxybenzoate (**II-14**)by the procedure described above for compound **II-28.** ^{1}H NMR ($CDCl_3$, 400 MHz) δ 7.25-7.41 (m, 34H), 5.10 (m, 12H), 4.41 (m, 4H), 3.77 (m, 4H), 3.65 (m, 8H).

V. General procedure for hydrogenolysis of carbohydrate compounds α, β-D-Glucopyranose pentakis[3,4,5-trihydroxybenzoate] II-19 and II-20

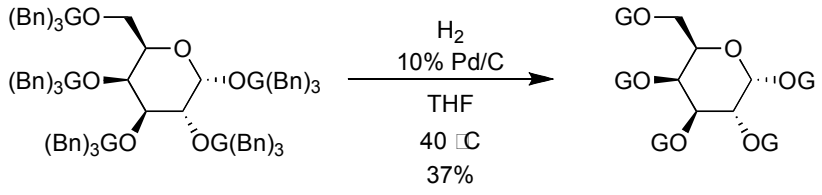


II-15 **II-19** **II-20**

β **α**

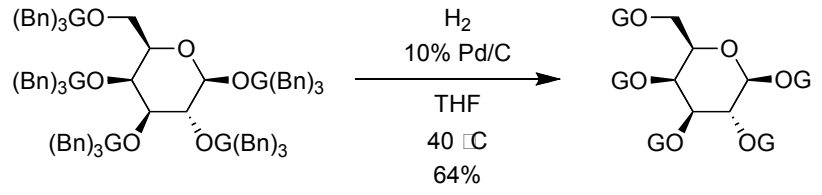
0.250 g (0.1 mmol) of D-glucopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] **(II-15**) was dissolved in 30 ml THF. A catalytic amount of 10 wt % (0.025 g, 0.23 mmol) palladium on carbon was suspended in the mixture, and a balloon containing H_2 was affixed to the stirring reaction flask. After 60 h at room temperature, the reaction mixture was filtered through celite and the filtrate was evaporated to provide the title compound in 22% yield (0.0179 g). ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.34–7.97 (m, 15H), 7.15 (s,

2H), 7.09 (s, 2H), 7.03 (s, 2H), 6.98 (s, 2H), 6.95 (s, 2H), 6.30 (d, *J* = 8.2 Hz, 1H), 5.99 (t, *J* = 9.6 Hz, 1H), 5.61 (m, 2H), 4.52 (m, 2H), 4.37 (dd, *J* = 12.8, 4.6 Hz, 1H).



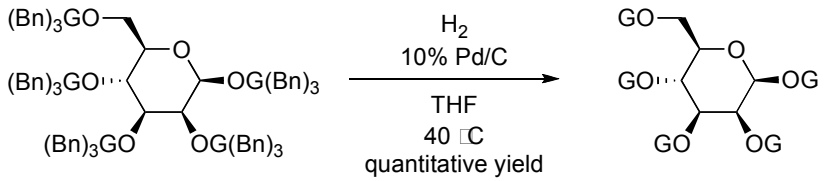
II-16 **II-21**

α-D-Galactopyranose pentakis[3,4,5-trihydroxybenzoate] II-21. Obtained in 37% yield from α-D-galactopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] (**II-16**) and hydrogen gas by the procedure described above for compounds **II-19 and II-20.** ^{1}H NMR (acetone-d_6, 400 MHz) δ 8.36–8.00 (m, 15H), 7.25 (s, 2H), 7.19 (s, 2H), 7.04 (s, 2H), 6.96 (m, 4H), 6.79 (d, *J* = 3.7 Hz, 1H), 6.05-5.99 (m, 2H), 5.78 (dd, *J* = 11.0, 3.7 Hz, 1H), 4.94 (m, 1H), 4.46 (dd, *J* = 11.0, 6.9 Hz, 1H), 4.24 (m, 1H).



II-17 **II-22**

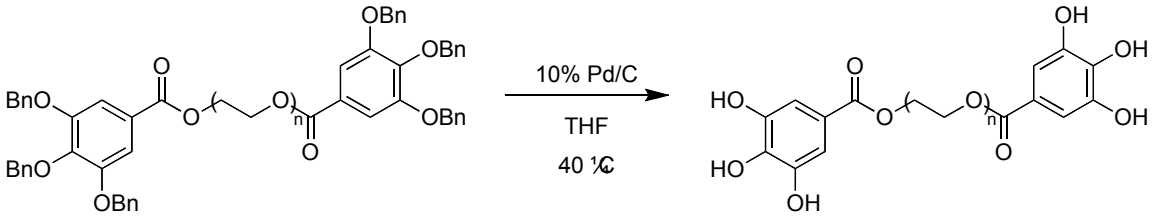
β-D-Galactopyranose pentakis[3,4,5-trihydroxybenzoate] II-22. Obtained in 64% yield from α-D-galactopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] (**II-17**) and hydrogen by the procedure described above for compounds **II-19 and II-20.** ^{1}H NMR (Acetone-d_6, 400 MHz) δ 8.18 (bs, 15H), 7.19 (s, 2H), 7.13 (s, 2H), 7.08 (s, 2H), 6.99 (s, 2H), 6.94 (s, 2H), 6.32 (d, *J* = 7.8 Hz, 1H), 5.92 (t *J* = 4.0 Hz, 1H), 5.83 (m, 2H), 4.75 (t, *J* = 8.0 Hz, 1H), 4.49 (m, 1H), 4.27 (m, 1H). IR (CH_2Cl_2) ν 3339, 1705, 1603, 1531, 1444, 1342, 1211, 1091, 1034, 871, 768.



II-18 **II-23**

β-D-Mannopyranose pentakis[3,4,5-trihydroxybenzoate] II-23. Obtained in quantitative yield from α-D-mannopyranose pentakis[3,4,5-tris(phenylmethoxy)-benzoate] (**II-18**) and hydrogen by the procedure described above for compounds **II-19 and II-20.** ^{1}H NMR (acetone-d6, 400 MHz) δ 8.46–7.97 (m, 15H), 7.29 (s, 2H), 7.20 (s, 2H), 7.13 (s, 2H), 7.08 (s, 2H), 6.96 (s, 2H), 6.46 (d, *J* = 1.4 Hz, 1H), 5.93 (t, *J* = 10.1 Hz, 1H), 5.85 (dd, *J* = 10.1, 3.2 Hz, 1H), 5.76 (m, 1H), 4.66 (m, 1H), 4.50 (dd, *J* = 10.1, 2.3 Hz, 1H), 4.44 (dd, *J* = 12.4,5.5, 1H).

VI. General procedure for hydrogenolysis of digalloyl compounds



II-28: n = 1 **II-30**: n = 3
II-29: n = 2 **II-31**: n = 4

II-32: n = 1 **II-34**: n = 3
II-33: n = 2 **II-35**: n = 4

1,2-Bis-galloyloxyethane II-32. 0.255 g (0.28 mmol) of di-*O*-(3,4,5-tribenzyloxybenzoyl)ethylene glycol (**II-28**) was dissolved in 25 ml THF. A catalytic amount of 10 wt% (0.025 g, 0.23 mmol) palladium on carbon was suspended in the mixture, and a balloon containing H_2 was affixed to the stirring reaction flask. After 18 h at room temperature, the reaction mixture was filtered through Celite and the filtrate was evaporated to provide the title compound in quantitative yield. ^{1}H NMR (acetone-d_6, 400

MHz) δ 8.19 (s, 4H), 8.03 (s, 2H), 7.12 (s, 4H), 4.52 (s, 4H).

Diethylene glycol, digallate II-33. Obtained in 44% yield from di-*O*-(3,4,5-tribenzyloxybenzoyl)diethylene glycol (**II-29**) and hydrogen gas by the procedure described above for compound **II-32.** ^{1}H NMR (Acetone-d_6, 400 MHz) δ 8.13 (bs, 6H), 7.12 (s, 4H), 4.31 (m, 4H), 3.76 (m, 4H), 3.65 (s, 4H). 13C NMR ($CDCl_3$, 400 Mhz), δ 205.68, 205.48, 128.46, 128.28, 128.06, 127.92, 127.74, 108.45, 29.23, 29.04, 28.85. IR (CH_2Cl_2) ν 3733, 3702, 3662, 3626, 3615, 3605, 2361, 2325, 1705, 1598, 1536, 1490, 1449, 1326, 1214, 1106, 1034, 687.

Triethylene glycol, digallate II-34. Obtained in 51% yield from di-*O*-(3,4,5-tribenzyloxybenzoyl)triethylene glycol (**II-30**) and hydrogen gas by the procedure described above for compound **II-32**. ^{1}H NMR (acetone-d6, 400 MHz) δ 8.13 (bs, 6H), 7.12 (s, 4H), 4.31 (m, 4H), 3.76 (m, 4H), 3.65 (s, 4H).

Tetraethylene glycol, digallate II-35. Obtained in 14% yield from di-*O*-(3,4,5-tribenzyloxybenzoyl)triethylene glycol (**II-31**) and hydrogen gas by the procedure described above for compound **II-32**. ^{1}H NMR (acetone-d6, 400 MHz) δ 8.25 (s, 6H), 7.12 (s, 4H), 4.31 (m, 4H), 3.74 (m, 4H), 3.60 (m, 8H). IR (CH_2Cl_2) ν 3854, 3627, 1694, 1612, 1536, 1449, 1343, 1232, 1036.

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