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Site-Selective *N*-Methylation of Resin Bound Terminal Amines And Borane Reduction / Iodine Work-up: Amino Acid Side Chain Compatibility and Solution Studies.

by

Carmen M. Laplante ©

A thesis submitted to the Faculty of Graduate Studies and Research in partial fulfillment of the requirements for the degree of Master of Science.

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Dr. Dennis Hall (Supervisor)

Dr. David Bundle

Dr. Leo Spyracopoulos

Dec 12/00

ABSTRACT

A methodology for site-selective mono-*N*-methylation of a resin bound primary amine has been developed based on a 1,2-carbon to nitrogen rearrangement of boron. Alkylation with excess pinacol chloromethane boronate followed by a subsequent repair rinse with hydrogen peroxide in a buffered cocktail solution gave the desired *N*-methyl-phenylalanine and valine substrates with >95% efficiency.

A borane reduction / buffered iodine work-up protocol has been applied to resin bound peptides and its compatibility with various side chain functionalities (Val, Phe, Tyr, Ser, Cys, Met, Trp) was indicated by the high yields and purities of the corresponding polyamines. Solution studies of the reduction / oxidative work-up protocol indicates that only one equivalent of iodine is required, along with all buffer components, and subsequently, a reasonable mechanistic pathway has been proposed.

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To my son Brett

It takes a child's mind

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LIST OF ABBREVIATIONS

[α]	specific rotation
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
AIBN	2,2'-azobisisobutyronitrile
Ala	alanine
Anhyd	anhydrous
APT	attached proton test
Ar	aryl
Asp	aspartic acid
Boc	butyloxycarbonyl
Bn	benzyl
Bp	boiling point
br	broad
Bu	butyl
t-Bu	tert. butyl
n-BuLi	*n*-butyl lithium
°C	degree Celsius
calcd	calculated

Cbz	benzyloxy carbonyl
CHA	cyclohexylamine salt
CH_2Cl_2	dichloromethane
CH_3CN	acetonitrile
Concd	concentrated
2-ClTrt	2-chlorotrityl
Cys	cysteine
d	doublet
d.	days
DEAD	diethyl azodicarboxylate
δ	chemical shifts in parts per million downfield from TMS
DIPEA	diisopropylethylamine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
Dod	4,4'-dimethoxydiphenylmethyl
EDT	ethanedithiol
Et_2O	diethyl ether
ES	electrospray
eq	equivalent
FAB	fast atom bombardment
Fmoc	9-fluorenylmethoxycarbonyl

g	grams(s)
Glu	glutamic Acid
Gly	glycine
h	hour(s)
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
His	histidine
HL	high load
HOBt	1-hydroxybenzotriazole
HPLC	high performance liquid chromatography
HR	high resolution
Hz	hertz
IR	infrared
J	coupling constant
Leu	leucine
Lys	lysine
LR	low resolution
m	multiplet
MBHA	methylbenzhydrylamine
Me	methyl
MeO	methoxy
MeOH	methanol

Met	methionine
min	minute(s)
mL	millilitre(s)
mmol	millimole
mp	melting point
MS	mass spectrometry
m/z	mass to charge ratio
NaOAc	sodium acetate
NMP	*N*-methylpyrrolidine
NMR	nuclear magnetic resonance
NO_2	nitro
Nu	nucleophile
OEt	ethoxy
PEG	polyethylene glycol
Ph	phenyl
Phe	phenylalanine
PMP	1,2,2,5,5-pentamethylpiperidine
pp	polypropylene
ppm	parts per million
i-Pr	isopropyl
PS	polystyrene
q	quartet

rf	retention factor
rt	room temperature
s	singlet
sec	second(s)
Ser	serine
SPPS	solid phase peptide synthesis
t	triplet
TEA	triethylamine
TFA	trifluoroacetic acid
TFE	trifluoroethanol
THF	tetrahydrofuran
TIPS	triisopropylsilane
TLC	thin layer chromatography
TMS	tetramethysilane
TFSOTf	trimethylsilyl triflate
Thr	threonine
TMSCl	trimethylsilyl chloride
Tos	tosyl
Trp	tryptophan
Trt	trityl
Val	valine
UV	ultraviolet

Chapter One

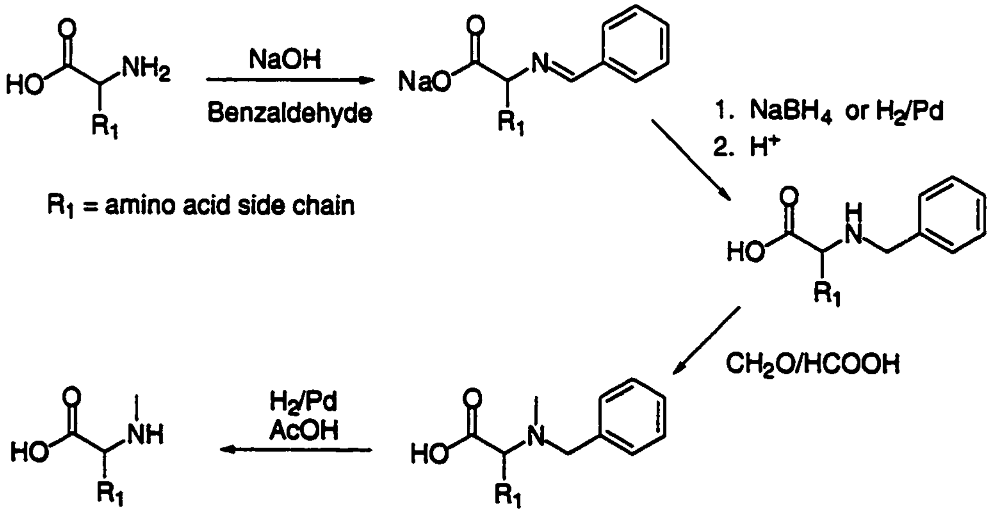
SITE-SELECTIVE *N*-METHYLATION OF RESIN BOUND TERMINAL AMINES

I. INTRODUCTION

Combinatorial chemistry has been an intensive area of research because it facilitates the rapid synthesis and screening of thousands of compounds.[1] With applications in many areas, including drug discovery, the demand for quantitative and clean methodologies for solid-phase synthesis has increased substantially. The use of clean methodologies on solid support would enable rapid construction of desired target molecules by avoiding tedious isolation and purification steps.[2] Another key advantage to solid-phase synthesis is that excess reagents can be used to drive the reactions to completion which then can be readily filtered off in the end.

Bioactive natural peptides containing *N*-methyl amino acid residues, such as Cyclosporine A[3] and HUN-7293,[4] are of considerable interest as drugs or as lead compounds for drug discovery.[5] *N*-Methylated peptides are useful models for structure – activity studies of peptide conformation[6] and may also demonstrate increased bioavailability over their peptide counterparts.[7] Some single *N*-methyl amino acid derivatives have been shown to be of biological interest.[8] Construction of *N*-methylated amino acids and selected peptide fragments of desired targets with existing solid-phase Fmoc/Boc chemistry is desirable. While controlled mono-*N*-methylation of a primary amino terminus appears straightforward, in practice it is very difficult to achieve and few *N*-methylated amino acids are commercially available.

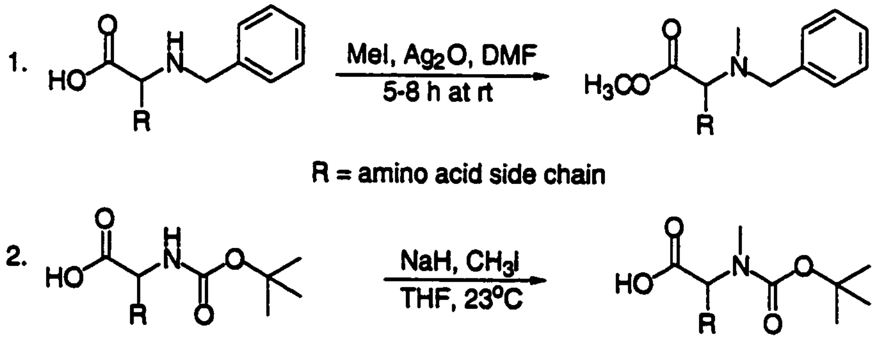
Many methods for mono-*N*-methylation of primary amines have been developed but none have yet been revealed as an ideal solution.[9] The obvious approach of alkylating primary amines with alkyl halides is complicated by formation of undesirable tertiary amines and quaternary ammonium salts.[10] Some of the more practical methodologies for mono-*N*-methylation of primary amines have been applied to selective *N*-methylation of amino acids. A solution-phase methodology whereby *N*-benzylamino acids can be methylated with formaldehyde-formic acid followed by reductive removal of the *N*-benzyl group has been presented (Scheme 1).[11] However, this approach requires further protection to a derivative suitable for peptide synthesis.



Scheme 1

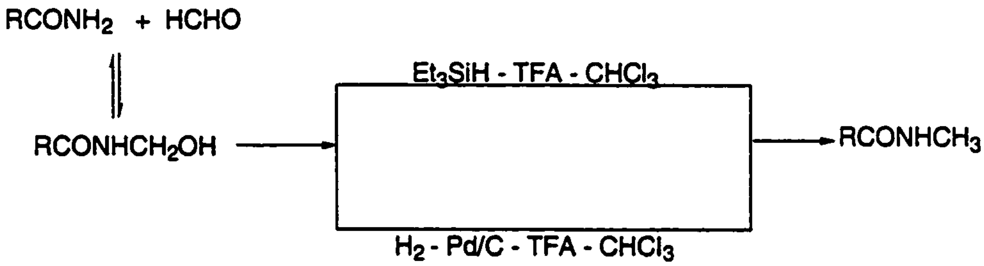
The synthesis of *N*-benzyloxycarbonyl-*N*-methylamino acid derivatives involves the use of methyl iodide and silver oxide in DMF (Scheme 2, Equation 1).[12] However, this methodology is limited to amino acid derivatives containing other functional groups not capable of undergoing methylation. Also, derivatives of cysteine or serine were reported to give a mixture of products including formation of dehydro-derivatives after treatment with methyl iodide and silver oxide.

N-*tert*-Butyloxycarbonyl-*N*-methylamino acids have been prepared by Benoiton with the use of NaH and MeI in THF at room temperature (Scheme 2, Equation 2).[13] This method would not be amenable to solid phase synthesis since *β*-elimination side reactions and selectivity on a growing peptide chain would be problematic.[14]



Scheme 2

N-Methylation of amides, lactams, and ureas can be achieved via methylol derivatives (Scheme 3).[15] As existing solid phase methods utilize the terminal amine for the amide bond formation step in peptide chain extension, this method is not appropriate. Furthermore, the use of trifluoroacetic acid renders this method incompatible with sensitive protecting groups and some resin linkers including the popular triphenylmethyl (trityl) resin linker.

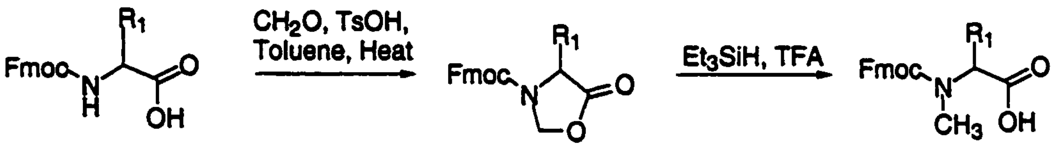


Scheme 3

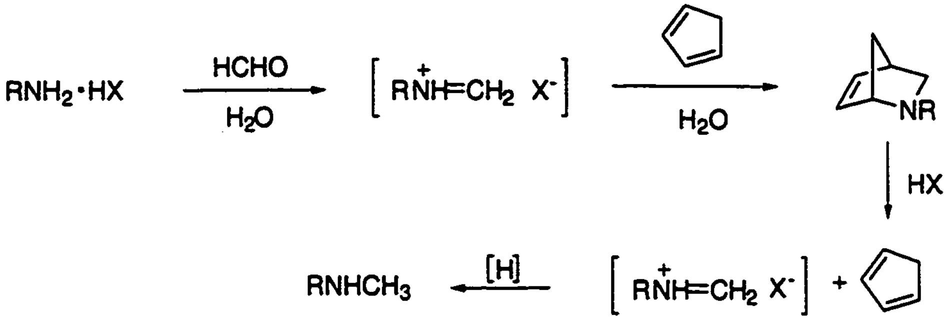
Fmoc protected *N*-alkyl amino acids have been prepared via reduction of oxazolidinones (Scheme 4) with reported 0.1% racemization.[16]

Retro Diels-Alder reactions of *N*-substituted 2-azanorbornenes followed by trapping of the incipient immonium ion with triethylsilane/TFA have been reported (Scheme 5).[17]

However, the use of heat and strong acids severely limit solid-phase applications of the two aforementioned methods.

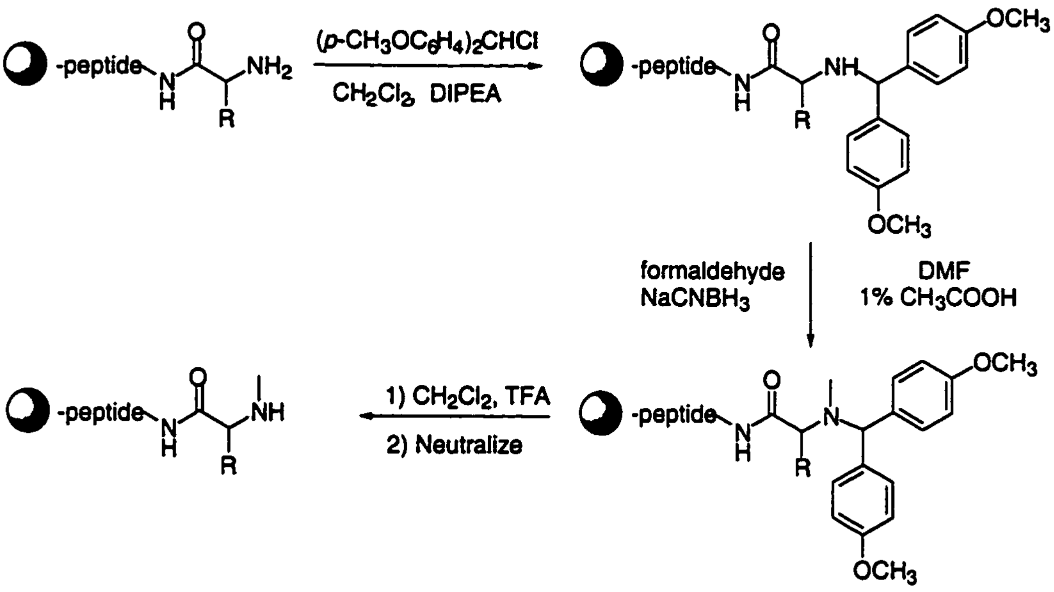


Scheme 4



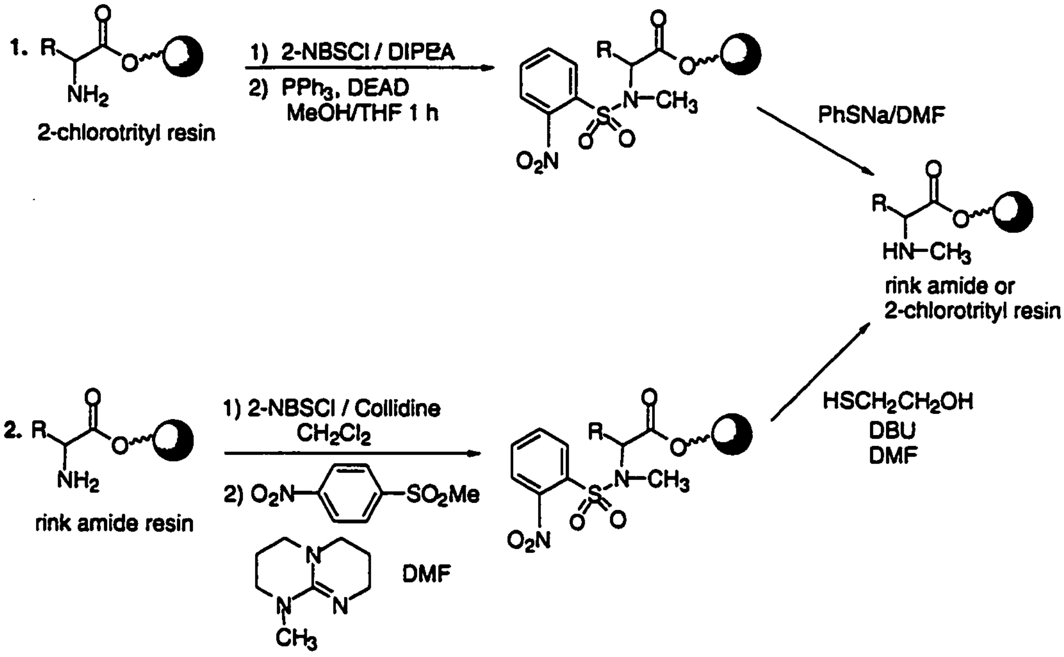
Scheme 5

A few multi-step solid phase-methods for site-selective *N*-methylation of peptides and amino acids on solid support have been reported. Protection of the amino terminus with the 4,4'-dimethoxydiphenylmethyl (Dod) group followed by reductive methylation has been presented (Scheme 6).[18] While the crude yields of peptides reported ranged from 56 to 99%, with low levels of side reactions, most substrates required 2 to 3 methylation cycles for completion. Also, this method is not amenable to Fmoc chemistry[18]– a key consideration as Carpino's Fmoc chemistry is extremely practical, thus popular, and is a strategy which enables the construction and cleavage of fully protected fragments.



Scheme 6

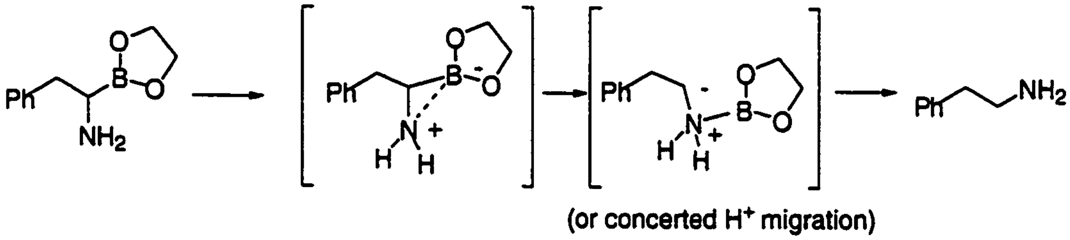
Another general approach utilizes applications of the Fukuyama amine protection / alkylation protocol.[10a] Solid-phase synthesis of Fmoc-*N*-methyl amino acids has been reported on 2-chlorotrityl resin under Mitsunobu - type conditions (Scheme 7, Equation 1).[19] Another group illustrated the practical utility of the modified Fukuyama protocol by demonstrating the *N*-methyl scanning of the thrombin receptor agonist peptide H_2N-SFLLRN-$CONH_2$ on rink amide resin (Scheme 7, Equation 2).[20] Methylation was reported to be 95% complete in all cases. Interestingly, these authors reported that they were unable to achieve any reaction at all under Mitsunobu - type conditions.



Scheme 7

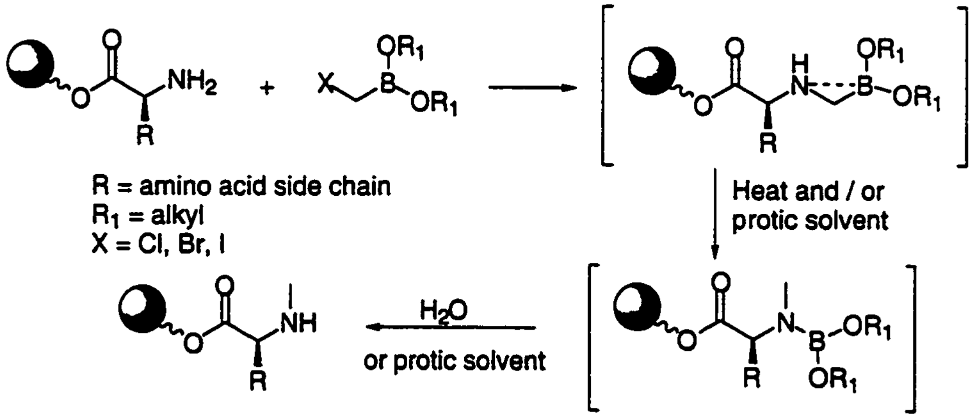
While these applications on solid support seem the most promising to date, they still require three synthetic steps[21] and the required alkylation conditions seem to vary amongst different resins. The search for universal, one step mono-methylation conditions for resin-bound or solution phase primary amines continues.

In initial attempts to construct alpha-acetimido boronic acid derivatives (the boron analogues of α-amino acids), Matteson and co-workers discovered a 1,2-carbon to nitrogen rearrangement of boron in aminomethylboronic esters (Scheme 8).[22] While the exact mechanism is unknown, it has been proposed by Matteson that the rearrangement likely occurs via an intramolecular nucleophilic attack of the amino group on boron followed by ring opening or proton migration. This proton migration might be concerted with ring opening. Tertiary amines are not known to undergo this rearrangement and it appears that a hydrogen attached to the amine nitrogen is required for this rearrangement to occur. Matteson also reported that this rearrangement is accelerated by heat and protic solvents.



Scheme 8

While this rearrangement was unfortunate with respect to Matteson's goals, we realized the possible application towards site-selective *N*-methylation of resin bound terminal amines. With the use of an alpha-haloboronic ester as an electrophile, it was expected that upon monoalkylation an intramolecular boron-to-nitrogen coordination bond would form and prevent further alkylation (Scheme 9). A protic work-up would promote the rearrangement to give the desired monomethylated terminal resin bound amine and therefore provide a quick, mild, and general approach to selective mono-*N*-methylation. In theory, such an approach would truly be remarkable considering the intensive past efforts towards the development of *N*-methylation methodologies. The first part of this thesis presents the optimization of reaction conditions towards the development of a *N*-methylation methodology for resin bound terminal primary amines based on Matteson's rearrangement.

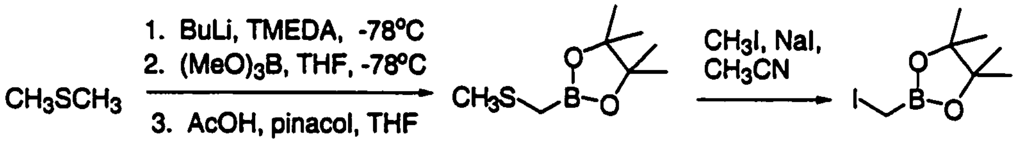


Scheme 9

II. RESULTS AND DISCUSSION

Initially, L-phenylalanine preloaded on Wang resin (1% DVB) was chosen as a model amino acid substrate. Pinacol iodomethane boronate was initially synthesized using Phillion's approach as shown in Scheme 10 and was used as the electrophile.[23]

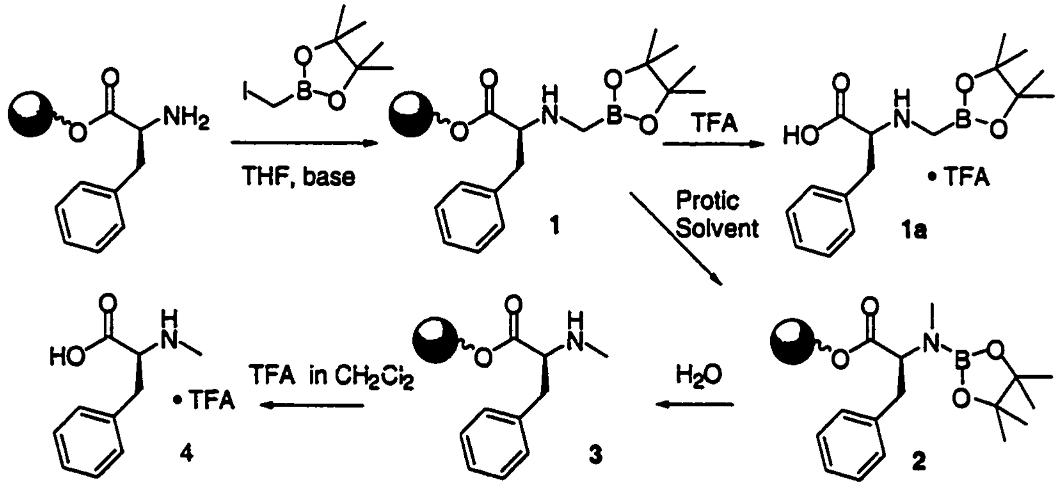
Synthetic Scheme for Pinacol Iodomethane Boronate



Scheme 10

As an initial experiment, a two hour long alkylation in THF with three equivalents of electrophile, no external base, and no protic solvent rinses was carried out to observe the initial alkylation product after cleavage of the product from solid support with trifluoroacetic acid (**1a**, Scheme 11). A large pinacol peak at δ 1.3 ppm and a methylene peak at δ 2.1 ppm in the ^{1}H NMR ($CDCl_3$) spectrum was observed. Low resolution MS (FAB) in glycerol showed a *m/z* of 306 (M + 1) which also suggested the presence of the monoalkylated but unrearranged amino methylboronate moiety (**1a**). It was established for immediate purposes that even though alkylation was not complete, a 4 : 1 THF : H_2O treatment of the resin at room temperature for ~11 hours prior to the trifluoroacetic acid promoted cleavage was sufficient to complete the rearrangement of **2** to the desired compound **3**, as indicated by the methyl singlet at δ 2.7 ppm in the ^1H NMR (CD_3OD) of the crude product **4**.

Selective-*N*-Methylation on Solid Support



Scheme 11

The first goal was to optimize the alkylation step. Organic bases were sought out that are generally readily protonated yet are relatively poor nucleophiles due to their steric bulk[24] as the goal was to avoid premature destruction of the electrophile by the base. In addition, the swelling of the hydrophobic polystyrene resin, solubility, and use of a mild approach are considerations that favour the use of relatively non-nucleophilic organic bases. As pinacol iodomethane boronate has been reported to form crystalline ammonium salts with tertiary amines,[23] experiments were then carried out where the electrophile (1.5 – 2.0 equivalence) was introduced half of an hour prior to the introduction of the base. While PMP and DIPEA gave comparable results, PMP was chosen for further trials primarily due to its reluctance to form quaternary salts and its higher pK_a (11.25).[24] It has since been determined that the rate of alkylation appears to be faster using cheaper DIPEA as the base rendering it a more appropriate choice.[25] By observing the disappearance of the doublet of doublets from the α-proton in the starting material at δ 4.2 ppm and the appearance of a pseudo-triplet at δ 4.1 ppm (mono-*N*-methylated phenylalanine) in the ^1H NMR (D_2O) spectra, the best alkylation result obtained was a 1 : 1 mixture of starting material and monomethylated product. In cases where more than one equivalent of base was used, several unexplained peaks were observed in the δ 2.6 to 4.5 ppm region of ^1H NMR (D_2O) spectrum. It was suspected that the resin bound primary amine binds the released hydroiodic acid and any subsequent exchange with the free amine in solution occurs too slowly for practical purposes and prevents further alkylation.

Several trials were conducted where PMP was introduced prior to the electrophile. It was then determined that the majority of the alkylation occurs within five minutes from the introduction of the electrophile (Table 1). In cases where more than one equivalent of base or more than three equivalents of electrophile were used, several peaks were observed in the ^1H NMR (D_2O) spectrum in the δ 2.5-4.5 ppm region, which we now know to represent rearranged dialkylated material (Figure 1, phenylalanine analogue of **7**). An experiment with two by five minute rounds of the alkylation conditions yielded the best result of 77% monoalkylation with the pinacol iodomethane boronate (entry 5). In all cases, a white, D_2O insoluble polymer-like material could be observed in the crude samples. Low resolution MS (FAB) in glycerol of this mystery material revealed a *m/z* peak of 394 with a boron isotopic distribution pattern. ^{1}H NMR (DMSO) of this substance indicated peaks in the aromatic region. These results, in combination with fairly consistent yields of over 100% suggested significant amounts of dialkylated but unrearranged material (Figure 1, phenylalanine analogue of **8**). The second alkylation would have to be faster than the carbon to nitrogen rearrangement of boron in order to account for the formation of this dialkylated material.

The inability to surpass the 77% monomethylation mark with the pinacol iodomethane boronate as the electrophile in combination with its high reactivity prompted exploration with the diisopropyl (chloromethyl) boronate as a possible substitute. This electrophile was synthesized according to a literature protocol (Scheme 12).[26] Repeating the conditions of the best result to date with the new electrophile yielded almost no alkylation product. Further experiments confirmed the expectation of a substantially slower alkylation rate with this chloro-analogue of the electrophile. A 75% monomethylation result was achieved with 5 : 1 electrophile : PMP stoichiometry at room temperature for 21.5 hours. The increase in the amount of electrophile employed may be a reflection of the purity (80-90% by

^{1}H NMR) of the starting diisopropyl (chloromethyl) boronate. Inconsistent results were obtained with this electrophile presumably due to its poor purity.

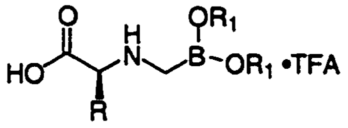
Table 1 : Exploratory *N*-Methylation Trials

Entry[1]	Pinacol iodomethane boronate eq.	PMP eq.	Solvent / Alkylation Time	Ratio of Starting Material : Mono-methylated Product[2] / Comments
1	3	1	DMF / 2 h	1 : 3
2	3	1	DMF / 5 minutes	1 : 2.2
3	5	2	THF / 2 h	1 : 1 / Several peaks in ^{1}H NMR at 2.5 – 2.9 ppm
4	10	1	DMF / 2 h	1 : 1.6 / Several peaks in ^{1}H NMR at 2.5 – 2.9 ppm
5	3	1	DMF / 2 rounds of 5 minutes each	1 : 3.4
6	1.1	5	THF / 2 h	1 : 1.2
7	3	1	CH_2Cl_2 / 2 h	1 : 1.7
8	3	1	Et_2O / 2 h	1 : 1.6
9	3	1	DMF / 5 rounds of 5 minutes each	1 : 3 / ^{1}H NMR spectrum was very messy

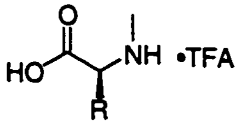
Base was added before the electrophile, work-up was 4 : 1 THF : H_2O for 11 + h and 90% TFA : CH_2Cl_2 cleavage off solid support was carried out for 2 h.

1. Preloaded Fmoc-Phe-Wang Resin was purchased from Novabiochem (0.40 mmol/g loading) and typical scale was ~ 50 mg of resin..
2. As determined by ^{1}H NMR integration of α-protons of D_2O soluble material.

Possible Alkylation Products

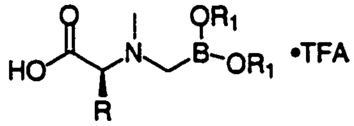


5 Monoalkylated
and unrearranged

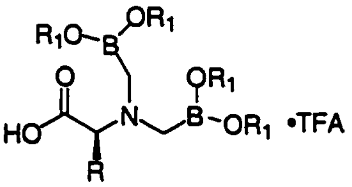


6 Monoalkylated
and rearranged

R = Amino acid side chain
R_1 = H, alkyl



7 Dialkylated
and rearranged



8 Dialkylated
and unrearranged

Figure 1

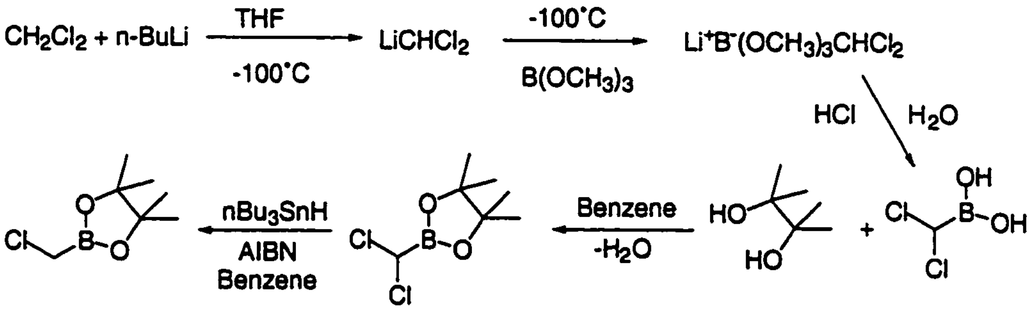
Synthetic Scheme for Diisopropyl Chloromethane Boronate

$$B(O\text{-}i\text{-}Pr)_3 + ICH_2Cl + n\text{-}BuLi \xrightarrow[\text{2. HCl in Et}_2\text{O, 10°C}]{\text{1. THF, -78°C}} ClCH_2B(O\text{-}i\text{-}Pr)_2$$

Scheme 12

Pinacol chloromethane boronate was then synthesized using Wuts approach (Scheme 13).[27] After the presented preliminary trials, another series of trials was undertaken in the presence of a protic solvent (to promote the rearrangement) using preloaded valine on Wang resin to determine if controlled mono-methylation could be achieved in one step (Table 2). Valine was chosen as the model compound due to the steric challenge that this amino acid imposes.

Synthetic Scheme for Pinacol Chloromethane Boronate



Scheme 13

Table 2 : 'One-Step' Alkylation Trials on Phe-Wang Resin

Entry[1]	Reaction Solvent	Crude Yield[2] %	Approximate % Starting material / % Monomethylated / % Dialkylated by ^{1}H NMR (CD_3OD)
1	4 : 1 DMF : MeOH	73	<5 / 85 / 10
2	9 : 1 DMSO : H_2O	100+	84 / 11 / <5
3	4.5 : 1 THF : MeOH	92	10 / 80 / 10
4	4.5 : 0.5 DMF : MeOH	73	<5 / 77 / 18
5	4.5 : 0.5 DMSO : MeOH	82	15 / 70 / 15
6	4 : 1 CH_2Cl_2 : MeOH	94	20 / 70 / 10

1. Resin was Fmoc-Phe-Wang (0.55 mmol/g loading from Novabiochem, 200-400 mesh size). All reactions were carried out with 2 eq. PMP and 3 eq. pinacol chloromethane boronate at rt for 22 – 24 h and typical scale was with ~50 mg of resin.
2. After TFA cleavage from solid support.

While success was achieved in limiting the amount of recovered starting material to less than 5% in some cases, there was significant amounts of dialkylated and rearranged material (Figure 1, valine analogue of 7) in the crude samples. From here onwards in this document, for simplicity, dialkylated material will refer to dialkylated and rearranged material. Peaks in the ^1H NMR spectrum (CD_3OD) of the crude material consisted of doublets at δ 2.1, 2.4, and 3.5 ppm indicated the presence of this impurity. A LRMS (low resolution mass spectrum) ES (MeOH) M + 1 peak of 218 indicated the methyl boronic ester derivative of the dialkylated product.

As the use of a 4 : 1 DMF : MeOH solvent system gave the best results, several trials with this solvent system were conducted in an attempt to minimize overalkylation utilizing the 'one pot' approach with no acid rinses (Table 3). With a protic solvent system, the rates of both the alkylation and the rearrangement were noticeably higher.

Table 3 : 'One-Step' Alkylation Trials on Val-Wang Resin

Entry[1]	Electrophile[2] eq	PMP eq	Alkylation Time (h)	Approximate % by ^1H NMR of Crude Overalkylation Product/ Monomethylation Product/ Starting Material
1	3.5	2	12	38 / 60 / 2
2	3.5	2	18	25 / 73 / 2
3	3	2	12	33 / 62 / 5
4	3	2	18	30 / 65 / 5
5	3	3	12	38 / 60 / 2
6	3	3	18	33 / 65 / 2
7	1.5	1.2	24	3 / 67 / 30
8	2.0	1.2	24	6 / 69 / 25
9	2.5	2	24	20 / 75 / 5
10	2.5	1.5	24	30 / 65 / 5

1. Novabiochem Fmoc-Val-wang resin (0.64 mmol/g loading). Typical scale was from ~30 – 150 mg of resin.
2. Chloromethane pinacol boronic ester.

In all cases, the reaction solvent was 4:1 DMF : MeOH and there were no acid rinses.

It should be noted that PMP•HCl acid salts are not soluble in DMF but are soluble in MeOH.[24] Salt build up on the resin during alkylation may be alleviated in the presence of MeOH and promote resin swelling and subsequently, reagent penetration. Different alkylation times and reagent ratios were explored. In cases where more than three equivalents of electrophile were employed, there was significant amounts of dialkylated material. The use of reduced amounts of electrophile for over longer periods of time resulted in significant amounts of remaining starting material. The use of the iodoanalogue resulted in an unrecognizable ^{1}H NMR spectra. In summary, overalkylation was unavoidable with the 'one pot' approach. Consequently, a 'two-step' approach was undertaken (Tables 4 and 5).

Table 4 : 'Two-Step' Alkylation Trials on Phe-Wang Resin

Entry[1]	Electrophile[2] eq	PMP eq/ mg	Alkylation Time h	Approximate % by ^{1}H NMR of Crude Overalkylated Product/ Monoalkylated Product/ Starting Material
1	3	2	24	12 / 54 / 24
2	4	2	24	14 / 66 / 20
3	4	2	6	10 / 65 / 25
4	3	2	6	6 / 70 / 24

1. Novabiochem Fmoc-Phe-Wang resin (0.55 mmol/g loading). Typical scale was ~50 mg of resin.
2. Pinacol chloromethane boronic ester

All alkylations were performed in DMF and were followed by a 24 h 4: 1 THF : H_2O work-up at rt.

Table 5 : 'Two-Step' Alkylation Trials on Val-Wang Resin

Entry[1]	Electrophile[2] eq	PMP eq	Alkylation Time h	Approximate % by ^{1}H NMR of Crude Overalkylated Product/ Monoalkylated Product/ Starting Material
1	4	2	24	28 / 61 / 5
2	3	2	24	24 / 71 / 5
3	3	3	24	22 / 74 / 4
4	2	1.5	24	15 / 68 / 17
5	2.5	1.5	24	27 / 64 / 9
6	2	1.3	24	4 / 68 / 28
7	2	1.1	24	5 / 70 / 25
8	3	0	24	10 / 60 / 30
9	3	0.5	24	14 / 60 / 26
10	3	3	10	5 / 70 / 25
11	2. 5	2	10	2 / 68 / 30
12	2	2	10	1 / 70 / 29

1. Novabiochem Fmoc-Val-Wang (0.64 mmol/g loading). Typical scale was ~50 mg of resin.
2. Pinacol chloromethane boronic ester

All alkylations were performed in DMF and were followed by a 24 h 4 : 1 THF : H_2O work-up at rt.

We wanted to determine if overalkylation could be minimized by first carrying out the alkylation in an aprotic reaction solvent to prevent rearrangement in the presence of excess electrophile and therefore avoid dialkylation. In principle, overalkylation is only possible if the B-N bond of the monoalkylated product is cleaved in the reaction mixture. Again, shorter reaction times or reduced amounts of electrophile resulted in significant amounts of starting material whereas longer reaction times resulted in significant amounts of overalkylation.

Several trials with aprotic solvent systems were carried out (Table 7). DMF was found to be a superior solvent in terms of rate of reaction. This finding is consistent with literature with respect to *N*-alkylation in general.[24] Unfortunately, the use of other solvents such as dichloromethane and THF resulted in a significantly slower reaction rate. Curiously, NMP, a common solvent used in solid support synthesis of peptides and similar to DMF rendered a reaction that was too slow for practical purposes. As stated previously, overalkylation is only possible if the B-N bond of the monoalkylated product is cleaved in the reaction mixture. At present, it is unclear whether this cleavage is caused by a neighboring resin bound amine, by a halide counterion from the base salt or by trace amounts of water as the reaction is not performed under an inert atmosphere.

Table 6 : Alkylation Trials with Various Aprotic Solvents

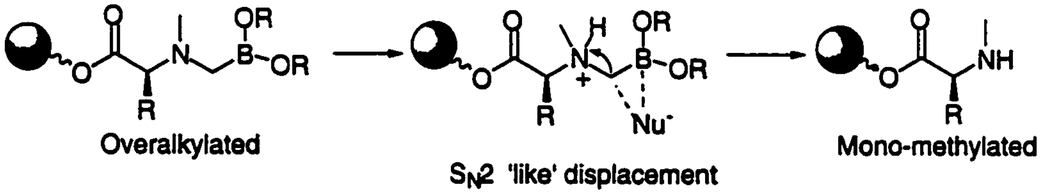
Entry[1]	Alkylation Solvent	% Crude Yield[2]	% Starting Material/ % Monomethylated Material/ % Dialkylated Material[3]
1	CH_2Cl_2	61	90 / 10 / trace
2	Toluene	93	95 / 5 / trace
3	THF	86	84 / 16 / trace
4	NMP	78	95 / 5 / trace
5	DMF	87	20 / 72 / 8

1. Fmoc-Val-Wang resin (0.73 mmol/g loading from RAPP Polymere (200-400 mesh size) and typical scale of ~50 mg) was charged with (after Fmoc removal) stated solvent, 3 eq. of pinacol chloromethane boronate, 2.2 eq. PMP for 9.5 h at 25°C. Resins were rinsed (~1 min. each) with 3 x 1 mL of stated alkylation solvent, 3 x 1 mL of 4 : 1 DMF : triethylamine, and 3 x 1 mL of 4 : 1 THF : MeOH. A 9.5 h treatment at 25°C of the resins with 4 : 1 THF : MeOH followed.
2. After 9 : 1 : 1 TFA : CH_2Cl_2 : H_2O cleavage from solid support.
3. Based on ^1H NMR integration (CD_3OD).

As it appeared that overalkylation was unavoidable, a 'repair solution' was sought after. A S_N2 'like' displacement of the α-amino boronate moiety was envisioned as there is literature precedent for similar processes in the (Scheme 14).[28]

Protonation of the amine with AcOH followed by displacement of the boronate moiety with the acetate anion was considered as a possible mild repair route. Ideally, such a repair solution could be employed in a resin washing operation. It was determined that a repair treatment of 24 h at room temperature with a 4 : 1 mixture of THF and 10% AcOH resulted in mono-*N*-methylated valine as the sole product though the yields were consistently lower than expected (see later). Control experiments with 4 : 1 THF : H_2O and THF : dry AcOH as 'repair solutions' indicated a significantly slower rate of repair, determining that both H_2O and AcOH were required components of the repair solution. Several other work-up trials (with and without water) were conducted in different solvents (Table 7).

Envisioned S_N2 'like' Displacement Repair Mechanism



Scheme 14

Table 7 : Exploration of 'Repair' Conditions in Different Solvents

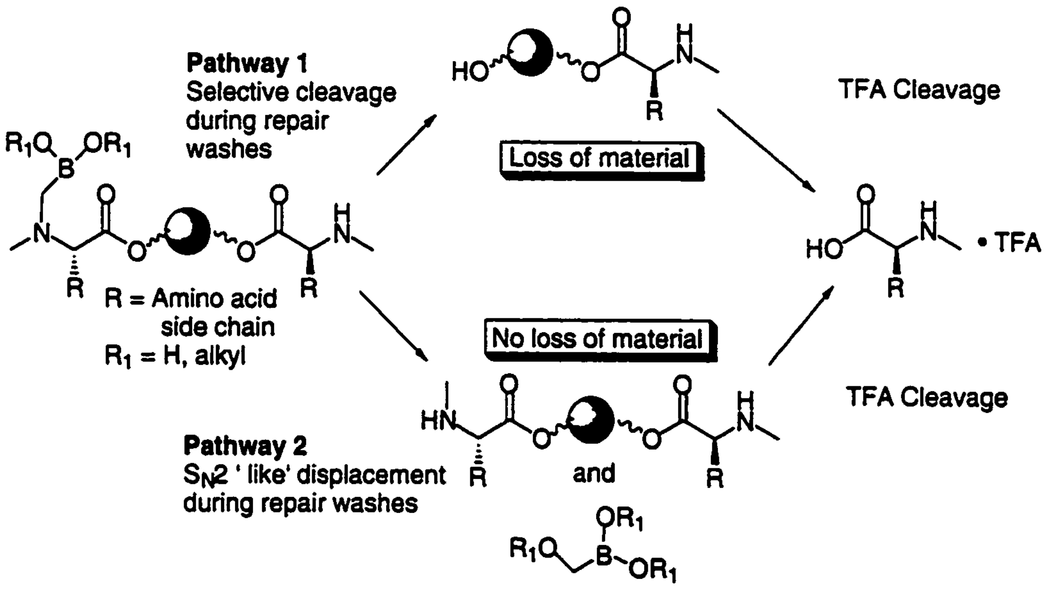
Entry[1]	Further Work-up Conditions (all at ~26°C for 24 h)	% Crude Yield[2]	% Starting Material/ % Monomethylated product/ % Dialkylated product[3]
1	AcOH (10 eq.) in 1 mL CH_2Cl_2	117	<5 / 72 / 23
2	AcOH (10 eq.) in 1 mL toluene	111	<5 / 71 / 24
3	1 mL of 4 : 1 DMF: 10% AcOH	113	<5 / 68 / 27
4	AcOH (20 eq.) in 1 mL CH_2Cl_2	109	<5 / 72 / 23
5	AcOH (30 eq.) in 1 mL CH_2Cl_2	107	<5 / 78 / 17
6	1 mL of 4 : 1 (1 : 1 : 1) THF / CH_2Cl_2 / DMF : 10% AcOH	89	<5 / 71 / 24
7	1 mL of (1 : 1 : 1) THF/ CH_2Cl_2/ DMF : AcOH (10eq.)	125	<5 / 71 / 24

1. Fmoc-Val-Wang resin (0.73 mmol/g loading from RAPP Polymere (200-400 mesh size)) 0.5755 g resin was alkylated (after Fmoc deprotection) with 3 eq. of chloromethane pinacol boronate ester and 2.2 eq. of PMP in 4 : 1 DMF : MeOH for 24 h at ~25°C. The resin was then rinsed with 3 x 5 mL DMF, 3 x 5 mL 4 : 1 THF: H_2O, 3 x 5mL MeOH, 3 x 5 mL CH_2Cl_2, dried, and then was split for further work-ups (all at ~26°C for 24 h) as illustrated.
2. After cleavage with 9 : 1 : 0.5 TFA : CH_2Cl_2 : H_2O cleavage from solid support and 12 + h drying under high vacuum.
3. Based on ^1H NMR (CD_3OD) integration of α-CH peaks at δ 3.5 and δ 3.8 ppm. (Dialkylated material was approximately 41 % present prior to any further work-up conditions.)

It was determined that 4 : 1 THF : aqueous acetic acid mixture was the repair solution of choice. As overalkylation was no longer a consideration with a repair solution in hand, the amount of electrophile and PMP employed was increased to 5 : 4.2 equivalents respectively for method generality purposes. As potential acid build up during the alkylation step is undesirable, the ratio of PMP was adjusted accordingly.

Two possible mechanistic pathways for the 'repair' step were initially considered. The first pathway involves activation of the linker's ester carbonyl by boron towards cleavage and the second pathway would be the originally envisioned S_N2 'like' displacement of the α-aminoboronate (Scheme 15).

Possible 'Repair' Pathways



Scheme 15

A control experiment with Fmoc-Phe-Wang resin subjected to a 4 : 1 THF : aqueous 10% AcOH solution for 24 h at room temperature was carried out and the 'repair' rinses were concentrated, dried and subsequently analyzed by ES-MS (MeOH) and ^{1}H NMR (CD_3OD). No evidence for the presence of the Fmoc-amino acid could be found indicating that a normal Wang ester linkage was stable to the repair conditions. Examples with overalkylated (by up to 50%) valine, prior to treatment with the repair solution, consistently rendered ~70% + yields after treatment with the repair solution and subsequent cleavage. These findings indicated that selective cleavage could not be the sole mechanistic repair pathway.

Utilizing commercially preloaded Wang-AA-Fmoc resins (200 – 400 mesh size) from RAPP Polymere, larger scale alkylations followed by treatment with repair solutions were carried out on a Quest semi-automated synthesizer at 25°C in an attempt to address the yield issue (Table 8).

Table 8 : Amino Acid Compatibility Studies With 'Two-Step' Alkylation Approach

Entry	Resin-AA-Fmoc[1] /Weight Used (g)	Dry Weight of Resin After Alkylation (g)/ New Loading[2] (mmol/g)	Weight of Resin Cleaved (g) / % Crude Yield
1	Ala / 0.49	0.42 / 0.82	0.20 / 34
2	Phe / 0.49	0.38 / 0.93	0.18 / 42
3	Glu(*t*-Bu) / 0.51	0.41 / 0.83	0.21 / 31[3]
4	Asp(*t*-Bu) / 0.48	0.38 / 0.99	0.18 / 33[3]
5	Lys(*t*-Boc) / 0.48	0.34 / 1.09	0.15 / 24[3]
6	Tyr(*t*-Bu) / 0.51	0.38 / 1.01	0.15 / 19[3]
7	Thr(*t*-Bu) / 0.51	0.44 / 0.73	0.20 / 61[3]
8	Met / 0.49	0.37 / 1.03	0.15 / 73
9	Ser(*t*-Bu) / 0.47	0.37 / 0.96	0.17 / 15[3]

1. All preloaded resins were purchased from RAPP Polymere (200-400 mesh size) and all alkylations were performed on a Quest Synthesizer. After Fmoc removal, all resins were charged with 10 mL of 4 : 1 DMF : MeOH followed by 4.2 eq. of PMP and 5 eq. of pinacol chloromethane boronate and left to agitate for 24 h at 25°C. A 24 h work-up with a 4 : 1 THF : 10% AcOH mixture followed. The resins were rinsed with 3 x 5 mL of DMF, 3 x 5 mL of 4 : 1 DMF : triethylamine, 3 x 5 mL MeOH, 3 x 5 mL of CH_2Cl_2 and then dried under high vacuum for 12 + h.
2. Based on original loading and mass balance.
3. After ether precipitation.

The percent crude yields (Table 8) were disappointingly low with evidence of dialkylated product in all examples with the exception of the phenylalanine analogue. It was not clear whether or not β-substituted amino acids like valine and threonine resulted in higher yields due to a lesser degree of overalkylation (and subsequently, less selective cleavage) or if the presumed chair-like transition state required for selective cleavage is sterically unfavorable and another 'repair' mechanisim is then favoured. Upon concentration and drying of the saved 'repair' solution and subsequent THF rinses, dialkylated material was identified by ^{1}H NMR in all cases. However, mass balance calculations indicated some unaccounted loss of material in all cases. The alkylation solution from the Wang-Phe-NHCH$_3$ trial was concentrated and dried and the presence of dialkylated material was identified by ^{1}H NMR and LRMS ES though it was difficult to quantify due to the presence of salts in the concentrated alkylation mixture. The disappointing low yields and the presence of dialkylated product in the rinses indicated that selective cleavage was occurring even during alkylation presumably through methanolysis of the linker. Attempts at using this alkylation/repair protocol on a different size Wang resin (100-200 mesh size) resulted in complete alkylation with the phenylalanine and valine analogues but little sign of any repair except when the repair step was carried out at 35 - 40°C. It is suspected that diffusion of water or acetic acid through the polystyrene matrix is responsible for the considerably slower rate of repair with the larger resin bead size.

Our attention turned to selective *N*-methylation of the third residue site of a tripeptide. As activation of the ester linkage by boron was not expected to occur primarily due to entropic considerations – the terminal residue would be too far away to activate the linker - other possible mechanistic pathways were expected to predominate. Tripeptides (Wang-AlaPheAla-NH_2 and Wang-AlaPheVal-NH_2) were constructed on 200-400 mesh size resin from RAPP Polymere. After removal of the terminal Fmoc group from the third residue, the resin was subjected to the same alkylation/repair conditions the same as in Table 8. After TFA cleavage, the resulting concentrated and dried crude material was subjected to ^1H NMR and LRMS ES analysis. The spectra of the crude indicated the predominant species to be the desired monomethylated product with approximately 20% and <5% dialkylated and starting material respectively. If a second or third mechanistic repair pathway was occurring, it was occurring too slowly for practical purposes.

Compound **10** (Scheme 16) was then constructed as solution phase model as a Wang-amino acid mimic (Figure 2) to provide insight into possible mechanistic pathways. If selective cleavage is the only mechanistic pathway, then compounds **12** and **13** were to be the expected products after treatment with a 4 : 1 THF : 10 % AcOH at room temperature for 24 hours. However, products **11**, **12** and **13** as well as starting material could be identified by ^1H NMR and LRMS ES (M + 1 peaks of 272.2, 222.1, 362.2 respectively) after the repair treatment providing evidence of the occurrence of at least a second mechanistic pathway (possible S_N2) other than the pathway corresponding to internal activation of the ester.

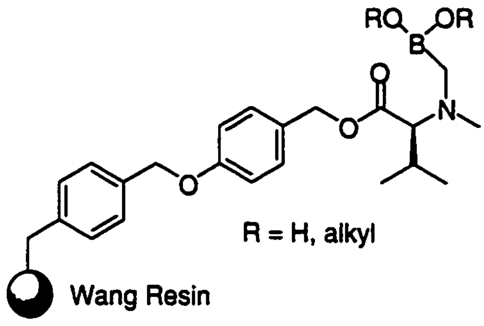
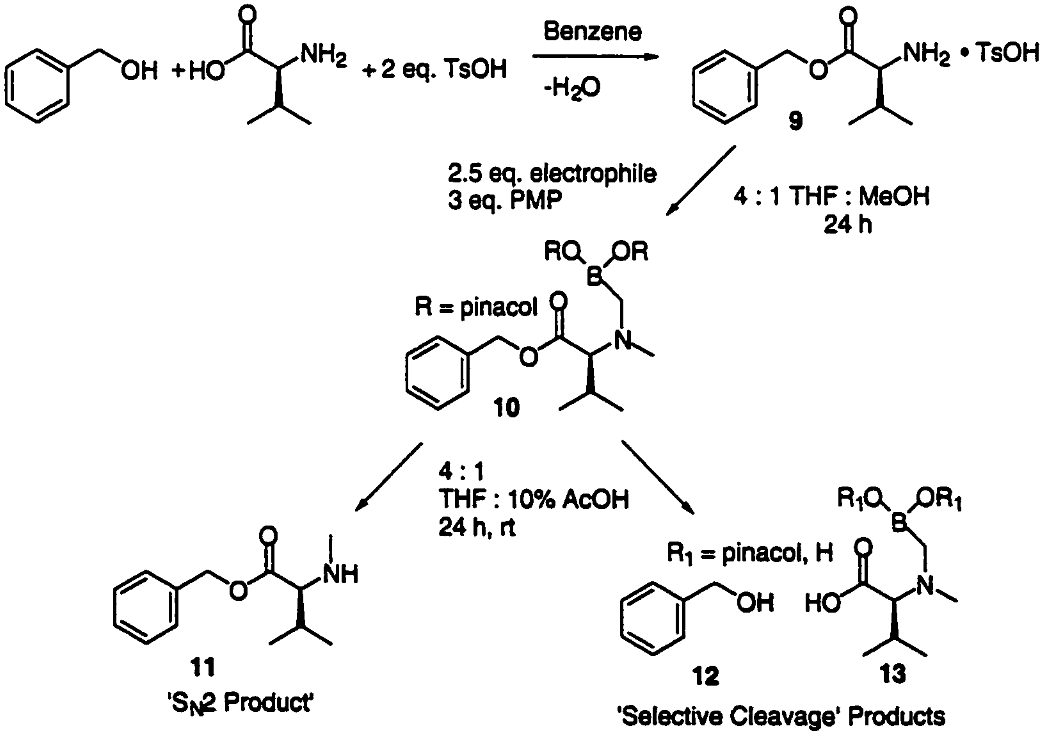


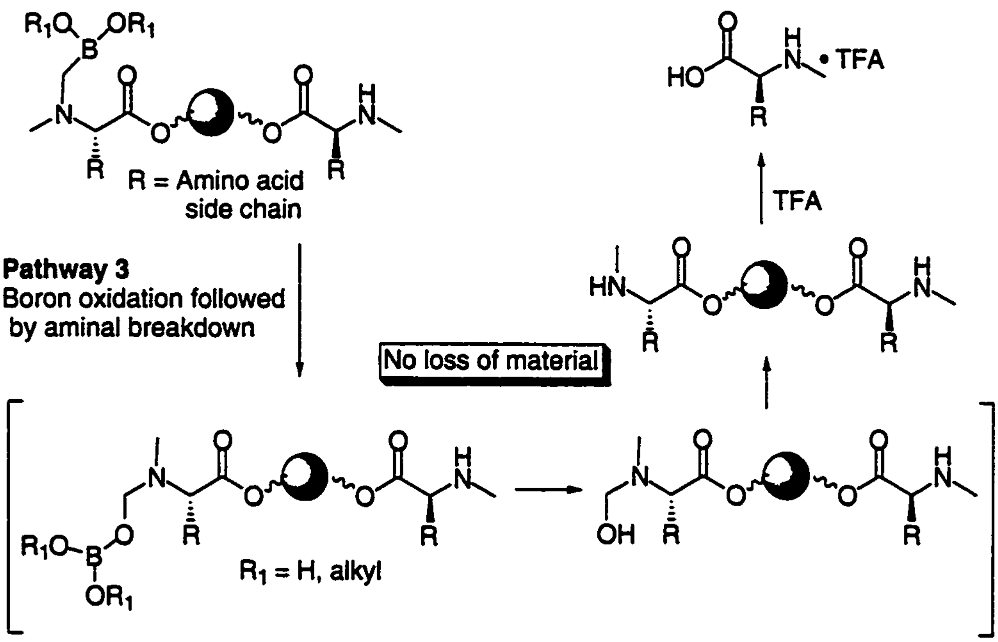
Figure 2 : Dialkylated Amino Acid on Wang Resin



Scheme 16 : Solution Phase Model Study

Unfortunately, because **11** and **12** could not be separated by column chromatography and because **10, 11,** and **13** have overlapping peaks in the ^{1}H NMR spectrum, a semi-quantitative analysis was not immediately available. However, the ^{1}H NMR spectrum of the concentrated crude 'repair' reaction mixture seemed to indicate that the selective cleavage products were predominant by the appearance of benzylic alcohol (**12**) protons at δ 4.6 ppm (CD_3OD). This disappointing result supported the previously presented results with significantly low yields in the solid-phase application.

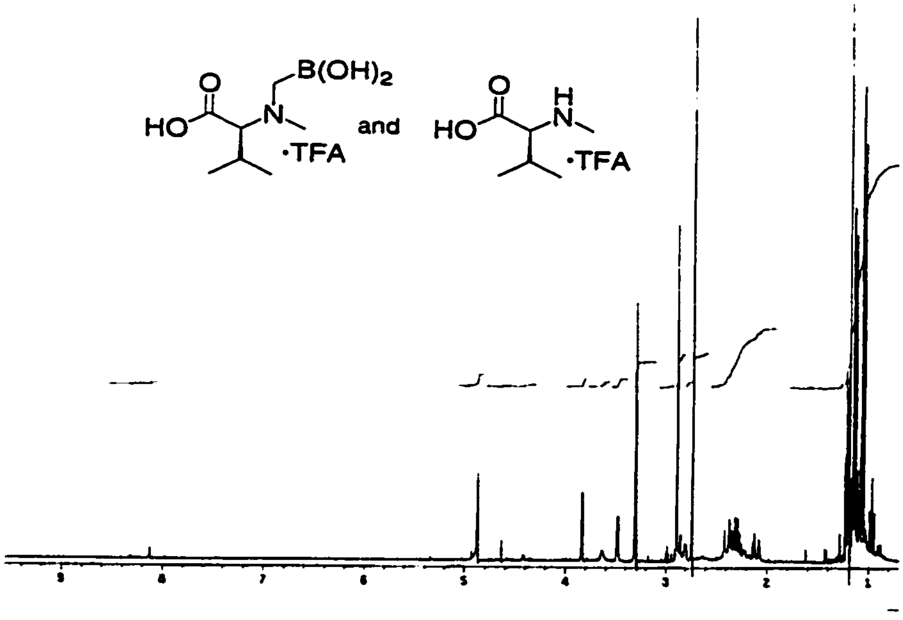
However, what was more insightful was the instability of the dialkylated amino acid ester **10**. Isolation and purification of **10** proved difficult in that it was not at all mobile or stable on silica. However, flash chromatography on Al_2O_3 provided reasonably pure material even though degradation on alumina TLC plates was clearly evident. Slow air oxidation of **10** was then suspected to be the cause of the instability of **10** and this led to the consideration of a third mechanistic repair pathway (Scheme 17).



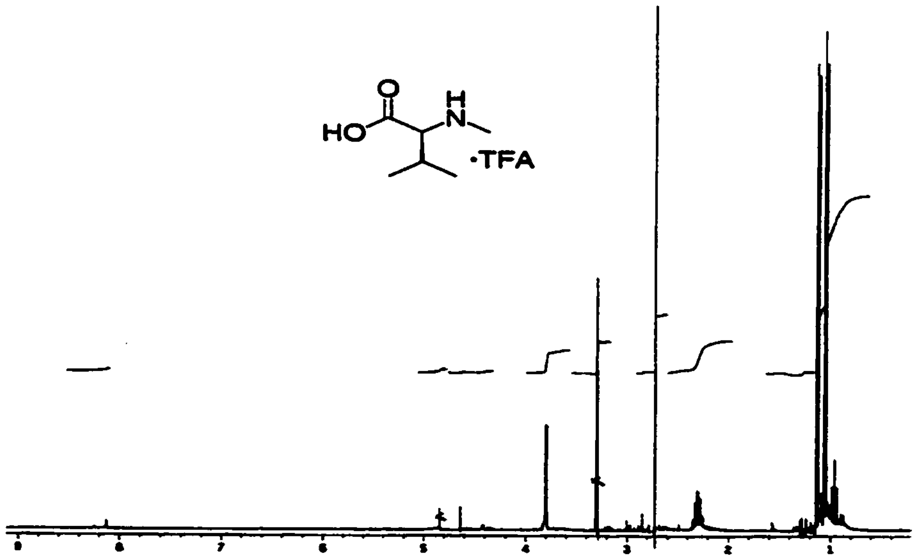
Scheme 17 : Peroxide 'Repair' Approach

The use of hydrogen peroxide to competitively promote this possible repair pathway was then a possible solution to circumvent other undesirable pathways such as selective cleavage. Overalkylated valine on Wang resin was subjected to three one minute 'repair rinses' of a 4 : 1 THF : pH 8 buffer and 20 + equivalents of H_2O_2[29] resulting in little to no detection of the dialkylated material signature peaks in the ^1H NMR (CD_3OD) of the crude mixture after cleavage from the solid support. Instead, new peaks could be observed. Singlets at 3.0 and 3.2 ppm and doublets at 4.3, 4.6 ppm indicated the formation of a new product. The downfield shift of the α-protons and general peak duplication in the ^1H NMR of the crude material indicated the presence of *N*-oxide product. The ES-MS (MeOH, positive mode) showed a M + 15 peak (*m/z* of 146) which also provides supporting evidence. *N*-Oxides can be synthesized with the use of peracids as well as sodium perborate from primary amines.[30] It was quickly determined that 1 to 5 minute rinses of the resin with the THF/buffer cocktail in the presence of 2.5 – 5 equivalents of H_2O_2 rendered a very clean desired product with little to no evidence of *N*-oxide formation (Figure 3). Longer exposure times (24 h) and especially higher concentrations of H_2O_2 promote the formation of the *N*-oxide derivative. Control experiments using 2.5 equivalents of peroxide for one hour did not result in significant amounts <5% of *N*-oxide. On the other hand, a one minute rinse with 1 equivalent of peroxide was not sufficient enough for complete repair. Another control experiment with a repair rinse of only 4 : 1 THF : pH 8 buffer resulted in no sign of repair indicating that the peroxide is the key requirement. The peroxide must preferentially attack the electron deficient boron under the buffered conditions and the subsequent breakdown of the resulting aminal intermediate must be quite rapid in aqueous solvent.

Before and After Peroxide 'Repair' Profile



A typical ^{1}H NMR (CD$_3$OD) spectrum of crude over-alkylated valine before 'repair' treatment with peroxide (on solid support) and after cleavage from solid support.



A typical ^{1}H NMR (CD$_3$OD) spectrum of crude *N*-methylated valine after 1- 5 min. 'repair' rinse with 5 eq. of peroxide (on solid support) and after cleavage from solid support.

Figure 3

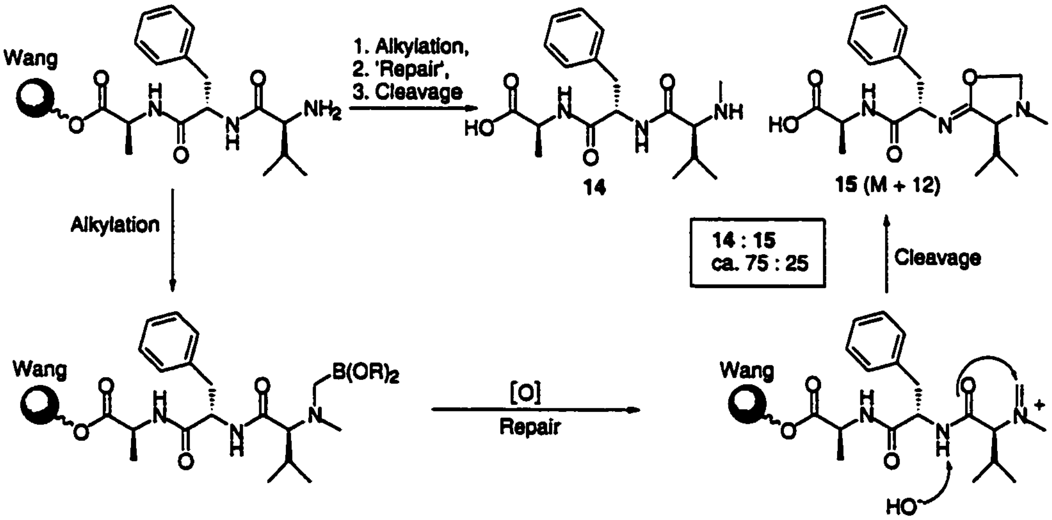
As evidence that the free monomethylated amine is the actual species truly generated on the solid support, the *N*-acetylated and *N*-Fmoc derivatives of valine were synthesized on solid support. Subsequential cleavage off the solid support and analysis of the crude mixtures indicated very respectable ^{1}H NMR (CD_3OD) and ES-MS spectra.

To address the yield issue and a possible mechanistic route, the phenylalanine derivative was chosen due to the previously observed low yield of 42% with the 4 : 1 THF : 10% AcOH repair solution approach. Utilizing DMF as the alkylation solvent in combination with the optimized protocol for the new repair solution of THF/pH 8 buffer/five equivalents of peroxide, a quantitative yield was obtained after TFA cleavage. Substitution of MeOH for the sterically hindered *tert*-amyl alcohol as a protic cosolvent in the alkylation step also resulted in a quantitative yield after the peroxide rinse and cleavage from solid support.[31] The ^{1}H NMR (CD_3OD) spectra of the crude product was very clean. The Fmoc derivative was synthesized on solid support, cleaved, chromatographed, and an optical rotation (15.7 mg, - 0.805°, 1 mL MeOH, 10 cm cell, $[\alpha]^{25}_{D}$ = - 51.3°) was obtained (the TLC indicated a UV active trace impurity present). The comparison to a commercial sample from Novabiochem (15.3 mg, - 0.925°, 1 mL MeOH, 10 cm cell, $[\alpha]^{25}_{D}$ = - 60.5°) indicated that little to no epimerization is occurring with this methodology although a formal chiral HPLC analysis with L and D isomers of an analogue of *N*-methylated phenylalanine would provide more substantial evidence.

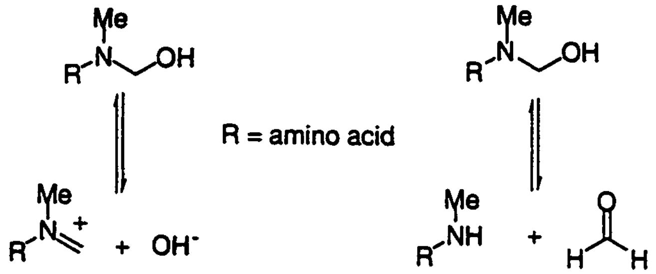
Construction of *N*-methylated amino acid residues on acid sensitive trityl resins would allow formation and cleavage of fully protected fragments. Applying the optimized protocol to trityl-val-NH_2[32] resulted in ~ 1 : 1 mixture of starting material and *N*-methylated valine after cleavage from the solid support. Use of excess of the chloro-electrophile resulted in no improvement. Use of the bromo- and iodo-electrophile analogues resulted in too many impurities including significant amounts of starting material. Steric hindrance due to the trityl linker is suspected to be the cause of underalkylation. The use of acid sensitive but less sterically demanding resins such as HMPB (Sasrin) resins will be explored in the future with the expectation of successfully synthesizing and cleaving fully protected fragments.

Upon applying the optimized protocol to the second residue site of Wang-Ala-Leu-NH_2, no material was obtained after cleavage from solid support.[33] However, material was found in the alkylation rinses. It is suspected that exposure of the resin bound peptide for 24 hours under basic conditions favours diketopiperazine formation and subsequent cleavage from the support during the alkylation step.

The optimized method was then applied to the third residue site on Wang-AlaPheVal-NH_2 (200-400 mesh size from RAPP Polymere). While the alkylation step was ~95 % complete, the repair with five equivalents of H_2O_2 for five minutes failed to completely eliminate what initially appeared to be some form of dialkylated but different material than previously seen (~25% dialkylated material by ^{1}H NMR (CD_3OD)). The ES-MS of the crude material indicated M + 1 (product) and M + 12 (impurity) peaks. The M + 12 peak is suspected to correspond to the cyclized terminal amino acid residue analogue (**15**, Scheme 18) that would presumably form via intramolecular capture of the transient imine. A similar result was obtained with Wang-Ala-Leu-Val-NH_2 as starting material (~70:30 mono-*N*-methylated tripeptide : undesired cycloadduct by ^{1}H NMR (CD_3OD)) when mono-*N*-methylation of the third residue site was attempted.[34] Formation of the imine would be a result of the aminal breaking down opposite of the desired way whereby formaldehyde is generated instead (Scheme 19).



Scheme 18 : Optimized Protocol with a Tripeptide



Scheme 19 : Directions of Aminal Breakdown

Attempts to limit the amount of this cycloadduct impurity at the oxidation step were carried out.[35] Oxidations performed at pH 6, 8, and 10 resulted in no change. Oxidation with the milder triethylamine *N*-oxide resulted in no change. Addition of ethylenediamine or hydroxylamine to the oxidation mixture as an attempt to trap the formaldehyde resulted in no significant change. These findings appeared to illustrate an unfortunate limitation to the methodology for solid-phase applications.

An experiment was carried out by Professor Hall to 'fix' the 'repair step'. After alkylation and the initial treatment with peroxide, the resin bound tripeptide (Wang-Ala-Leu-Val-NX_2) was treated with a 4 : 1 THF : pH 10 buffer mixture containing ten equivalents of morpholine for two hours at room temperature in an attempt to trap the transient imine. Subsequent breakdown of the resulting aminal would hopefully proceed in predominantly one direction. After cleavage of the tripeptide off of solid support and subsequent identification by ^{1}H NMR, the product appeared to be the desired mono-*N*-methylated tripeptide. A single peak at *m/z*

316.3 (M + 1) in LRMS ES spectrum was observed with no sign of the corresponding cycloadduct impurity (M + 12). However, peak duplication in the ^1H NMR spectrum suggested the presence of diastereomers that presumably formed due to epimerization during the 'repair of the repair' step. Future attempts at a more general 'repair' approach on solid support will combine the peroxide treatment in the presence of morpholine at either a lower pH or shorter rinse times.

III. CONCLUSION

In conclusion, this methodology represents the first 'one-step' approach to selective mono-*N*-methylation of resin bound amino acids whereby mono-*N*-methylation can be achieved to greater than 95% completion. It represents a unique and original approach to a very old synthetic problem. While the method currently appears to be limited to the first residue site of the resin bound peptide, the overall mildness of this approach should be compatible with a wide variety of functional groups as well as a variety of resins. The preliminary results from the 'repair of the repair' approach beyond the first residue site with morpholine appear promising for further optimization studies. The electrophile is not yet commercially available, however, several synthetic methods exist for the synthesis of α-haloboronic esters from cheap, commercially available starting materials.

IV. EXPERIMENTAL PROCEDURES

General Methods: All processes involving air or moisture sensitive reactants were done under an atmosphere of dry nitrogen using oven-dried glassware. Reagents and solvents were reagent grade and used as supplied unless otherwise stated. Tetrahydrofuran (THF) was distilled over sodium and benzophenone under a nitrogen atmosphere. Benzene, dichloromethane, methanol were dried and distilled over calcium hydride under a nitrogen atmosphere. Ether was dried and distilled over lithium aluminum hydride under a nitrogen atmosphere. Triethylamine was distilled over potassium hydroxide. Acetic anhydride was distilled under nitrogen. Aldrich spectrochemical grade TFA was dried and distilled over P_2O_5. Anhydrous DMF was purchased from Aldrich. Distilled water was used as is from the departmental tap source. All amino acid derivatives were purchased from Novabiochem and used as received unless stated otherwise. Isoleucine was purchased from Sigma. The K_2PO_4 / NaOH pH 8 buffer was purchased from Fisher Scientific and the 30 % H_2O_2 solution was purchased from ACP Chemicals Inc. Resins were purchased from RAPP Polymere. All glassware employed in solid-phase reactions had been silanized (treatment with 10% TMSCl in toluene for over 12 h) and dried. Polypropylene filter vessels (pp vessels) were obtained from Bio-Rad. Reactions on solid support were performed in peptide flasks or polypropylene (pp) vessels, with vortexing on a mechanical wrist shaker or vortexer and all rinses are ~1 minute each with vortexing unless stated otherwise. Yields of crude cleaved compounds are based upon the starting loading level of commercial resins. Solvent evaporation was performed under reduced pressure below 40 °C using a Büchi rotary evaporator. High vacuum drying refers to at least 12 h at rt under 0.1 torr atmosphere unless stated otherwise.

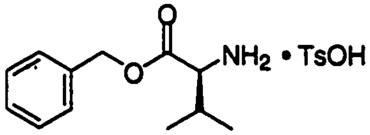
Optical rotations were measured on a Perkin–Elmer 241 polarimeter in 1 or 10 cm cells. Cast refers to the evaporation of a solution on a NaCl plate. Mass spectra (MS) were recorded on a Kratos AEIMS-50 high resolution mass spectrometer (HRMS), and AEI MS-9 fast atom bombardment (FAB) or a Hewlett Packard 1100 for electrospray instruments. Infrared spectra (IR) were recorded on a Nicolet 7199 FT-IR spectrometer. Nuclear magnetic resonance (NMR) spectra were obtained on Bruker AMR-200, AM-300 (for ^{11}B and ^{13}C), and Varian i300 (for ^{1}H) instruments. ^{11}B samples were recorded in WILMAD quartz tubes and were externally referenced to $BF_3•Et_2O$. ^{1}H NMR chemical shifts are reported in parts per million (ppm) downfield relative to tetramethylsilane (TMS) using the residual solvent resonance as the reference: $CHCl_3$, δ 7.24; HDO, δ 4.72; CHD_2OD. δ 3.30. ^{13}C NMR shifts are reported relative to: $CHCl_3$, δ 77.0; CHD_2OD, δ 49.0. ^{1}H NMR data are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; and m, multiplet), coupling constant(s) in Hertz (Hz) with a ± 0.5 Hz accuracy, number of protons, and assignment. When appropriate, the multiplicity is preceded by br, indicating that the singlet was broad.

As *N*-methylated valine and *N*-methylated phenylalanine and their Fmoc derivatives are commercially available, full characterization was not carried out and only the general preparation of *N*-methylamino acids is stated below. The *N*-methylated amino acid derivatives synthesized as stated in Table 8 (page 25) were not clean enough for proper characterization. Compound **10** was synthesized as stated below, however, full characterization was not performed due to its poor purity.

Typical synthesis of resin-bound *N*-methylamino acids. A sample of *N*-Fmoc-phenylalanine Wang resin (0.56 g, 0.72 mmol/g substitution) in a pp vessel was treated with 20% piperidine/DMF (5 mL, 3 min.; then 5 mL, 30 min). After rinsing with DMF (5 × 5 mL), the resin was suspended in dry DMF (10 mL) and PMP (0.31 mL, 1.7 mmol) was added and vortexed (0.5 min). Pinacol chloromethane boronate (0.34 mL, 2.0 mmol) was charged and the subsequent mixture was allowed to vortex for 22 h at rt after which time the resin was washed with DMF and 4 : 1 THF : pH 8 buffer solution (3 × 5 mL each). A solution of 4 : 1 THF : pH 8 buffer (10 mL) was charged followed by 30% H_2O_2 (0.12 mL, 5.0 eq) and vortexed for 1 to 2 min after which time the resin was washed successively with 4 : 1 THF : H_2O, 4 : 1 DMF : TEA, MeOH, and CH_2Cl_2 (3 × 5 mL each). The resin was dried under high vacuum for > 12 h to give 0.48 g resin (new loading of 85 mmol/g).

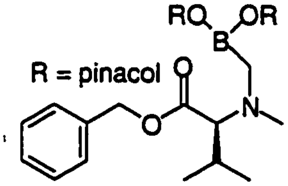
Typical cleavage of *N*-methylated amino acids off of solid support. Resin bound *N*-methylphenylalanine was prepared as stated above. CH_2Cl_2 (0.2 mL) and 1.8 mL

TFA, and 50 μL H_2O of a cleavage cocktail was charged to a portion of the resin (0.20 g, 0.17 mmol) in a 5 mL round bottom flask, and the mixture was gently stirred for 2 h at rt. The resin was filtered and rinsed forward through a glass wool plug with 5 × 1 mL CH_2CL_2, 1 mL TFA, and 1 mL CH_2Cl_2. The filtrate was concentrated under reduced pressure and then placed under high vacuum for >12 h. An off-white solid (52 mg) was obtained for a crude yield of 104%. D_2O soluble material was subjected to ^1H NMR analysis. ^1H NMR (D_2O) δ 7.4 (m, 5 H, H-Ph), 4.1 (apparent t, J = 6.2, 1 H, α-CH), 3.3 (m, 2 H, $-CH_2Ph$), 2.7 (s, 3 H, $-NCH_3$); LRMS ES in MeOH for $C_{10}H_{13}NO_2$ found 180.1 (M + 1).



Compound 9

Benzyl-*L*-valinate *p*-toluenesulfonate salt (9). Benzyl alcohol (8.6 mL, 0.083 mol), *p*-toluenesulfonic acid (6.33 g, 0.038 mol) and L-valine (1.95 g, 0.017 mol, purchased from Schwarz/Mann) were charged to 100 mL of benzene. The solution was left to reflux for 20 h in the presence of a Dean-Stark apparatus. The solution was then concentrated down under reduced pressure. The resulting residue was charged with 100 mL of Et_2O. The resulting slurry was filtered through a glass fritted funnel followed by 2 × 20 mL Et_2O rinses. The isolated white solids were further slurry washed in 100 mL of Et_2O for 1 h at rt. The solids were filtered off as before and placed under high vacuum for 24 h +. A white solid (5.98 g) was obtained resulting in a quantitative yield. ^1H NMR (CD_3OD) δ 7.70 (d, *J* = 8.0, 2 H, $-SO_2$-Ph-H); 7.38 (m, 5 H, benzyl-H), 7.21 (d, *J* = 7.9, 2 H, tosyl-H), 5.27 (apparent q, *J* = 11.9, 2 H , Ph-**CH_2**-), 3.93 (d, *J* = 4.5, 1 H, αCH), 2.35 (s, 3 H, Ph-**CH_3**), 2.25 (m, 1 H, -**CH**(CH_3)$_2$), 1.98 (d, *J* = 7.0, 6 H, -CH(**CH_3**)$_2$).



Compound 10

Benzyl *N*-methyl-*N*-(methyl pinacol boronic ester)-*L*-valinate (10). Pinacol chloromethane boronate (2.0 mL, 0.012 mol) was charged to a solution of compound 9 (1.7 g, 0.049 mol) and PMP (2.7 mL, 0.015mol) in 20 mL of 4: 1 THF : MeOH. The solution was stirred for 24 h at rt after which 0.43 ml DIPEA was charged. The reaction mixture was concentrated down under reduced pressure. The resulting residue was charged with 40 mL of Et_2O and the resulting solids were filtered off. The filtate was charged with 30 mL of hexanes and then concentrated under reduced pressure. The resulting slurry was charged with 15 mL of hexanes and then the solvent layer was decanted off and concentated under reduced pressure. The crude residue (1.38 g) was subjected to flash chromatography on a column prepared with 30 g of aluminum oxide (Aldrich-neutral-standard grade (%5 activated), ~ 150 mesh, 58 Å) and 100% CH_2Cl_2. The column was run with 50 mL CH_2Cl_2 and then 100 mL of 98 : 2 CH_2Cl_2 : MeOH. A clear light yellow oil (0.85 g) was obtained to give a yield of 57 %. ^{1}H NMR (CD_3OD) δ 7.32 (m, 5 H, Ph), 5.12 (s, 2 H, Ph-**CH_2**-), 2.93 (d, *J* = 10.5, 1 H, αCH). 2.35 (d. *J* = 16.5, 1 H, -NCH_2B-), 2.31 (s, 3 H, -NCH_3), 2.08 (d, *J* = 16.5, 1 H, -NCH_2B-), 1.99 (m, 1 H, -**CH**(CH_3)$_2$), 1.22 (s, 12 H, -$C(CH_3)_2C(CH_3)_2$-), 0.98 (d, *J* = 6.6, 3 H, -CH_3), 0.82 (d, *J* = 6.6, 3 H, -CH_3); LRMS ES in MeOH for $C_{20}H_{32}NO_4B$ found 362.1 (M^++H).

V. NOTES AND REFERENCES

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(32) Experiment carried out by Prof. D.G. Hall.

(33) Experiment carried out by Prof. D.G. Hall.

(34) Experiment carried out by Prof. D.G. Hall.

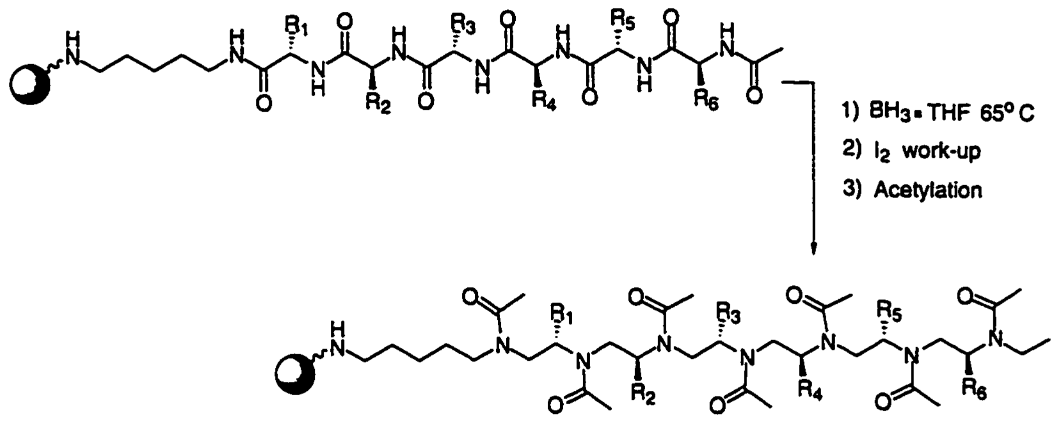
(35) Experiments carried out by Prof. D.G. Hall.

Chapter Two

Borane Reduction / Iodine Work-up: Amino Acid Side Chain Compatibility and Solution Studies.

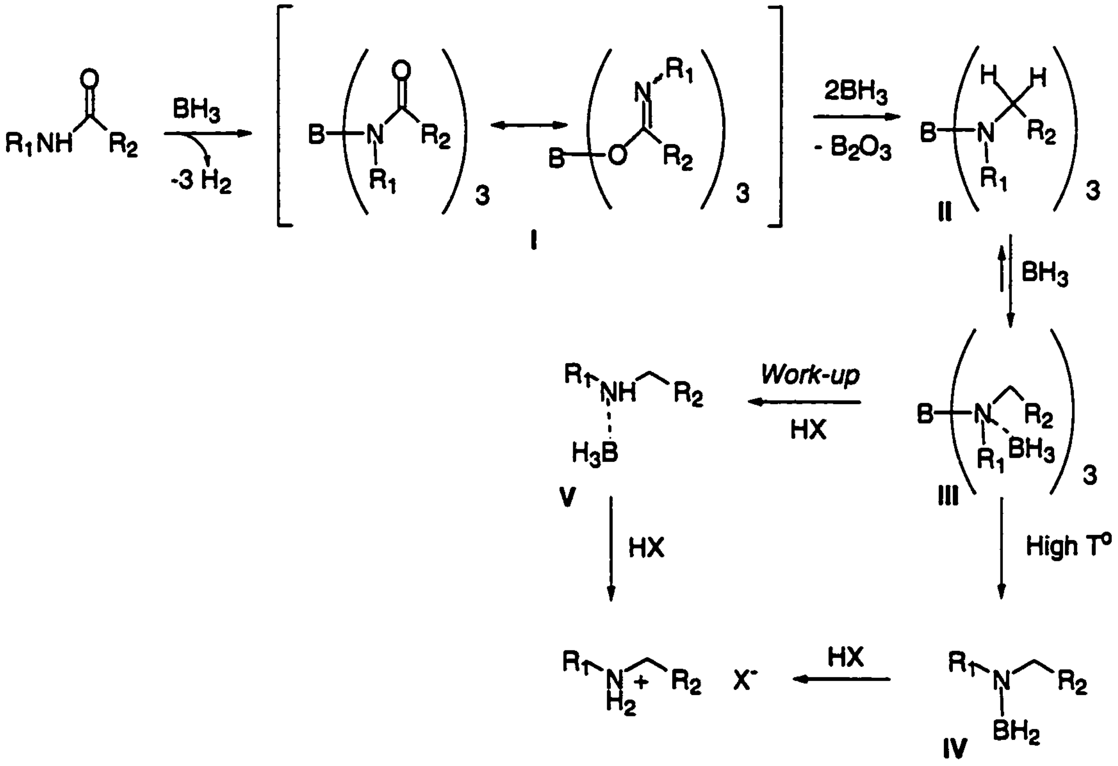
I. INTRODUCTION

In the second part of this thesis, the selective solid-phase synthesis of amine containing compounds, namely polyamines, is addressed. The development of a mild and practical method for solid-phase synthesis of polyamines[1] could serve towards library synthesis of unnatural biopolymers. Oligocarbamates,[2] oligoureas,[3] oligosulfonamides,[4] peptoids,[5] and beta-peptides[6] are just a few examples of the unnatural biopolymers that have been developed by other various methodologies. The solid-phase synthesis of polyamines would provide a practical approach to oligo-*N*-acylaziridines – a new class of unnatural biopolymers that have the distinct feature of the carbonyl function being 'exo' to the biopolymer backbone (Scheme 2.1). They are based on tertiary amides and are expected to demonstrate a relatively long *in vivo* lifetime by offering a high resistance to hydrolysis and enzymatic proteolysis.[7] Incorporation of diverse functionalities around a chiral backbone should encourage the existence of organized secondary structures such as helices.[8] The complete lack of backbone hydrogen bonds should provide interesting chemical properties compared to their 'natural' peptide counterparts. The use of commercially available and functionally diverse amino acids, in combination with a highly efficient synthesis, could readily allow for the creation of diverse chemical libraries of 'exo-peptides'.



Scheme 2.1

The reduction of secondary amides with borane has been reported and the mechanism that has been proposed is illustrated in Scheme 2.2.[9] Excess borane is often required for the reaction to go to completion due to the formation of a stable borane-amine aminoborane adduct **III**. While protocols have been developed to limit the amount of required borane,[10] excess borane used in solid-phase chemistry is utilized to drive the reaction to completion. The breakdown of the resulting borane-amine adducts **V** usually requires strong acidic[11] or basic[12] work-ups under extended reaction times and high temperatures.[13] These work-ups under harsh conditions would limit applications on solid support. The use of iodine for the titration of borane–amine adducts has been reported[14] and its modification for solid-phase synthesis with a buffered solvent system was developed by our group.[15] The scope and potential for oligoamines and subsequent libraries of 'exo-peptides' would greatly be expanded if the borane reduction with the mild oxidative work-up is compatible with various amino acid side chain functionalities. In the second part of this thesis, amino acid side chain compatibility with this method is examined and control experiments are carried out to gain insight into the mechanistic pathway of the oxidative work-up. Solution and solid phase applications will also be illustrated and discussed.

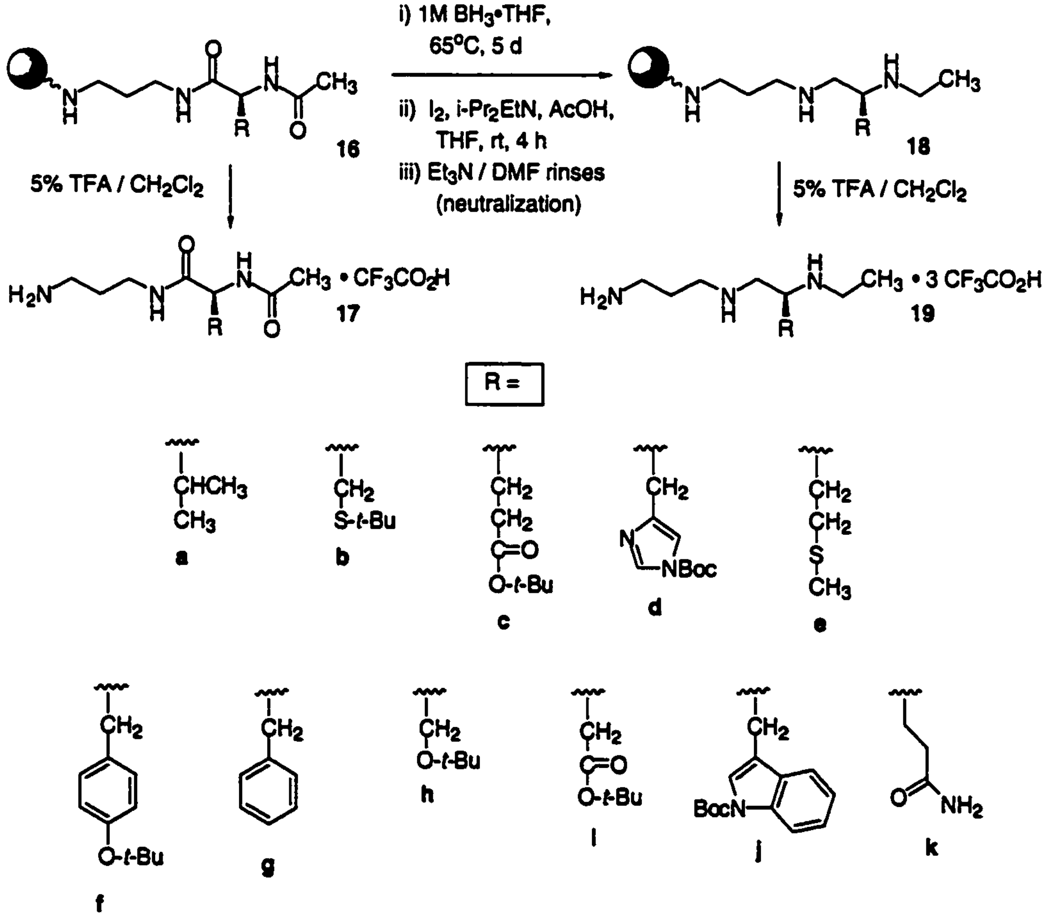


Scheme 2.2

II. RESULTS AND DISCUSSION

Section 1: Amino Acid Side Chain Compatibility With Borane Reduction / Iodine Work-up

The original long term goal, as previously stated in the introduction, is construction of 'exo-peptides' via peptide reduction chemistry on a solid support. Thus, determining the amino acid side chain compatibility with a borane reduction/iodine work-up is pertinent to future studies. Amino acids were attached to trityl resin via a propyldiamine linker, and the resulting terminal amines were acetylated with acetic anhydride. The resulting model peptides on solid support were subjected to exhaustive borane reduction, for five days at 65°C, followed by iodine (two equivalents per amide bond) in a 5 : 2 : 1 THF : AcOH : DIPEA cocktail mixture (Scheme 2.3). After cleavage from the solid support with trifluoroacetic acid in dichloromethane, with added diisopropylsilane or ethanedithiol as scavengers when necessary, the resulting triamines were examined by ^{1}H NMR, ^{13}C NMR, and ES-MS analysis. The ES-MS (MeOH) spectra of almost all examples showed M+12 and M+24 peaks with boron isotopic distribution patterns that were indicative of the presence of some boron containing impurity. However, these impurities were present in small quantities as they were not clearly evident by ^{1}H and ^{13}C NMR and insight into side chain compatibility was still obtained with most examples. It has since been established for the borane reduction/iodine work-up protocol that the use of five equivalents of iodine per amide bond is preferable in order to eliminate such impurities.[16] What was perhaps the most impressive result among the residues tested, was obtaining the fully intact *t*-butyl protected cysteinyl triamine **19b** (Table 2.1). As iodine oxidation of cysteinyl residues with various sulfhydryl protecting groups is a common approach to disulfide bond formation,[17] the fact that the fully intact triamine was obtained is a strong indicator to the mildness of the iodine/buffered work-up conditions. The methionine and serine



Scheme 2.3: Functional Groups Tested for Compatibility

Table 2.1: Functional Groups Tested for Compatibility

Entry	Peptide substrate	Triamine[a] product	Crude yield[b] (%)[l]	Purity (%)[c]
1	(Val) **16a**	**19a**	79	>90
2	(Phe) **16g**	**19g**	75	95
3	(*t*-Bu-Tyr)**16f**	**19f**	70	>90[d]
4	(*t*-Bu-Ser)**16h**	**19h**	61	90[e]
5	(*t*-Bu-Cys)**16b**	**19b**	79	>95
6	(Met) **16e**	**19e**	90	90
7	(*t*-Bu-Asp) **16i**	**19i**	81[f]	-
8	(*t*-Bu-Glu) **16c**	**19c**	79[f]	-
9	(Boc-Trp) **16j**	**19j**	79	95
10	(Boc-His) **16d**	**19d**	-	-
11	(Gln) **16k**[g]	**19k**	95[h]	>80[h]

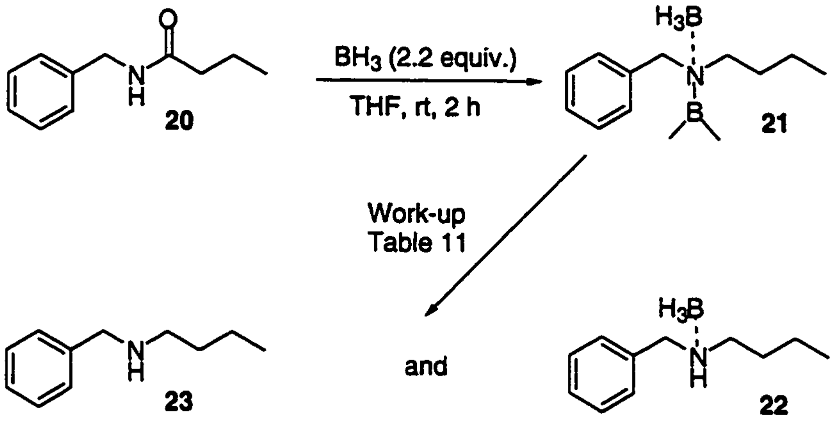
[a] General reaction conditions: typical scale 0.4 g resin 6, 1M BH_3, 65°C, 1-5 d; work-up: 2-3 equiv.I_2 in THF-AcOH-DIPEA 5 : 2 : 1. [b] Non-optimized yields after cleavage from resin (5% TFA/CH_2CL_2 and scavengers) and one round of precipitation with ether. [c] Purity was estimated by ^{13}C NMR (peak height comparison between peaks from products 19 and unknown ones). [d] Significant loss of the *t*-Bu group was observed in the resin cleavage operation so it was characterized as a free phenol. [e] A 5-10% loss of the *t*-Bu group was observed. [f] The ester side chain undergoes modifications whereby a mixture of the corresponding *t*-butyl ether and primary alcohol is obtained. [g] The glutamine side chain undergoes a modification to the corresponding primary amine. Experiment carried out by Dan Kopac. [h] Crude yield and purity (no ether precipitation).

analogue triamines **19e** and **19h** were also obtained in a good yield and quality. The aromatic triamines of tyrosine, phenylalanine, and tryptophan analogues (**19f**, **19g**, **19j**) yielded the expected products with no evidence of any iodine addition to the ring systems. Triamines with glutamate and aspartate residues **19c** and **19i** were obtained as variable mixtures of their corresponding esters and ethers. This is not surprising since a few specific examples of the reduction of hindered esters to ethers by borane have been reported in the literature.[18,19] The crude histidine analogue triamine **19d** was obtained as a mixture and consisted of the desired compound plus several unidentified products. It is unclear whether this mixture is due to the borane reduction or the iodine work-up, although an example of a histidine residue remaining intact after borane reduction on a solid support has been reported.[19] The hindered valine analogue triamine **19a** was obtained fully reduced and in good yield.

Section 2:

Solution Studies Towards Mechanistic Insight of the Iodine Work-up

As trifluoroacetic acid is extremely effective in cleaving boron-amine adducts,[20] it was necessary to determine that the free amines are generated on solid support (as a result of the iodine work-up) and not upon product cleavage off the solid support with trifluoroacetic acid. This was necessary to determine for future applications whereby the free amine is needed for 'on-bead' screening. In order to gain insight into the mechanism, efficiency and role of iodine in the work-up, several solution phase trials were carried out using a model secondary amide (Scheme 2.4, Table 2.2). For accurate ^{1}H NMR identification and comparison purposes, the amine product **23** (entry 1) and the corresponding borane-amine adduct **22** were synthesized independently by Tim Chan.



Scheme 2.4: Solution Studies with Model Secondary Amide

Table 2.2: Solution Studies with Model Secondary Amide

Entry #	Conditions[1]	Temp (°C)	Time (h)	Ratio **22** : **23** (%)[2]
1	acidic work-up (aq. 1N HCl)	rt	2	0 : 100[3]
2	no work-up	rt	2	80 : 20
3	1 : 2: 5 DIPEA/AcOH/THF	rt	2	60 : 40
4	same solvent, 0.5 eq. I_2	rt	2	30 : 70
5	same solvent, 1.0 eq. I_2	rt	2	0 : 100
6	MeOH / THF	rt	2	80 : 20[3]
7	1 : 2 :5 DIPEA / MeOH / THF	rt	2	80 : 20[3]
8	same solvent, 1.0 eq. I_2	rt	2	75 : 25[3]
9	excess TFA, THF	rt	2	0 : 100[3]
10	aq. conc. NaOH, THF	rt	20	75 : 25[3]
11	1 : 9 DIPEA / THF	rt	2	85 : 15[3]
12	excess morpholine, THF	rt	2	0 : 100

1. General conditions: model amide **20** was reduced with 2.2 eq. BH_3, 65°C, 3-4 h; then the indicated work-up is applied, followed by addition of aqueous base and thiosulfate (if necessary), followed by extractions with diethyl ether.
2. Measured by comparison of integrals of representative signals from the ^1H NMR spectra of the crude reaction mixture (estimated error: 5%)
3. Experiments carried out by Tim Chan

As sodium hydroxide was used in the basic extraction conditions to recover crude materials, a control experiment with such a work-up and only the basic extraction was carried out (entry 2). It was determined that approximately 20% of the borane-amine adducts were cleaved.

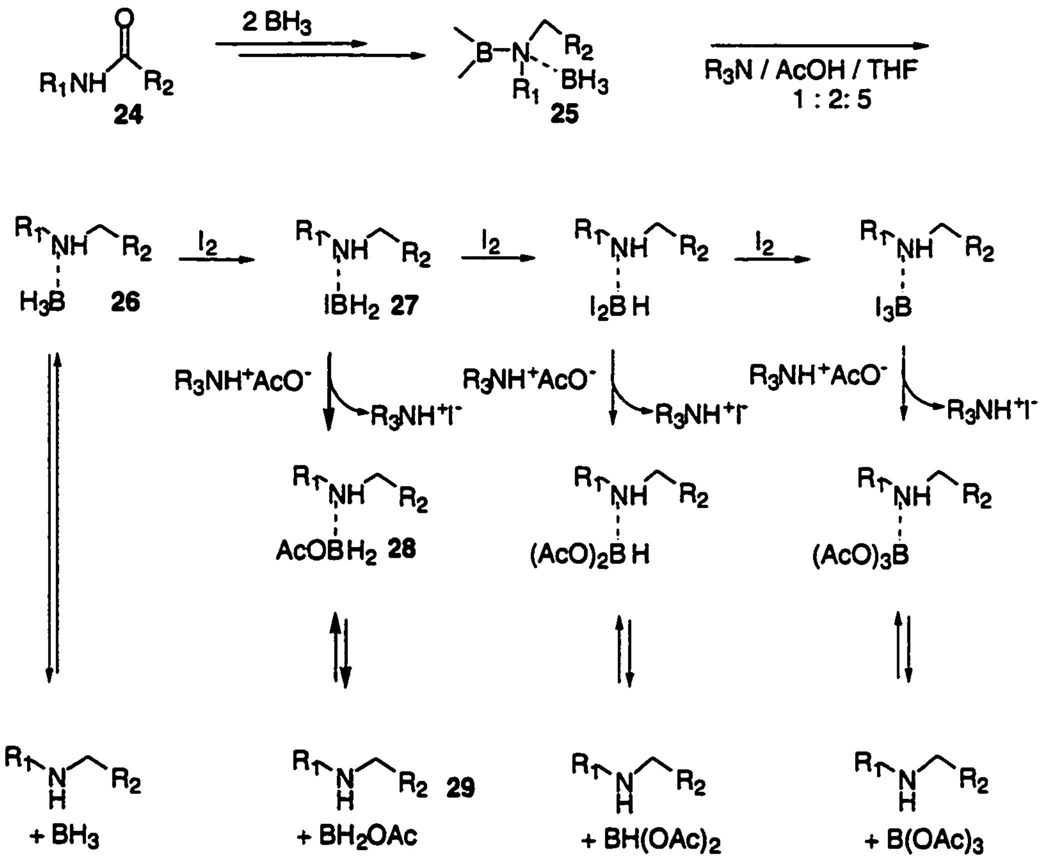
The control work-up with 1 : 2 : 5 mixture of DIPEA : acetic acid : THF for two hours without iodine (entry 3) resulted in approximately 40% cleavage of the borane-amine adduct **22**. It appears that the buffer solution alone can account for some background cleavage (~20%). However, considerably more sodium hydroxide (and consequently more sodium chloride) is required in the work-up to overcome the buffered system in solution to obtain the pH >11 that is required prior to carrying out extractions.

The work-up trials with 0.5 and one equivalent of iodine gave 70% and complete cleavage to **23** respectively. While it was determined that for a solution phase application only one equivalent of iodine per amide bond is required to completely cleave any boron-amine adducts, excess amounts of iodine are required for solid-phase applications in order to complete the work-up in four hours.

As exchange reactions of borane-amine adducts with excess external amines is deemed possible from the literature,[19] trials with DIPEA and morpholine were carried out (entries 7 and 12 respectively). While DIPEA appeared to render no cleavage above the background cleavage, the use of morpholine rendered solely the desired compound **23**. It is unclear if the same conditions at room temperature for 3-4 hours would be as effective on solid support. It should be noted that under extended reaction times at higher temperatures, piperidine has been reported to breakup boron amine adducts on solid phase media with work-up conditions at 65°C for >12 hours.[21,22]

Background cleavage renders the quantification of iodine enhanced cleavage of the borane-amine adduct difficult. What is conclusive is that iodine is a required element for the cleavage of the borane-amine adduct in a reasonable period of time in the solution trials and one equivalent of iodine appears to be sufficient.

Based on the results of the solution trials, a possible mechanistic pathway for the iodine work-up with one equivalent of iodine is proposed which involves all of the components in the buffered work-up solution (Scheme 2.5). The large excess of acetate anion in the buffer solution provides a source of an oxygen-based charged nucleophile which can partake in two roles: 1) the acetate anion very likely cleaves the aminoborane unit of adduct **25** to give **26**; 2) the acetate anion displaces the monoiodide in **27** via a S_N2 type reaction to form the acetoxyborane adduct **28**.[23] Dissociation of the acetoxyborane adduct is expected to be more facile than the monoiodoborane adduct due to backbonding of oxygen onto boron and thereby weakening the boron-nitrogen coordination bond.[24] The acetic acid could provide a



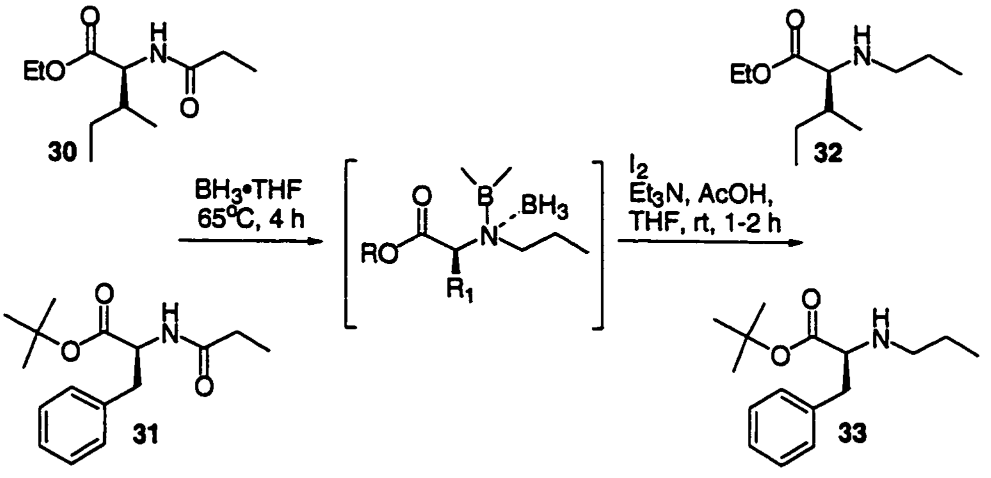
Scheme 2.5: Hypothesized Mechanistic Pathway of Buffered Iodine Work-up

general protic source to irreversibly trap the free amine (**29**). Trials with MeOH as a substitute protic source (entries 6-8, Table 2.2) resulted in no cleavage of the borane-amine adduct over the indicated background providing evidence for the criteria of sufficiently charged nucleophilic/acidic species in the work-up procedure. The large excess of DIPEA could trap any hydroiodic acid released in the process.

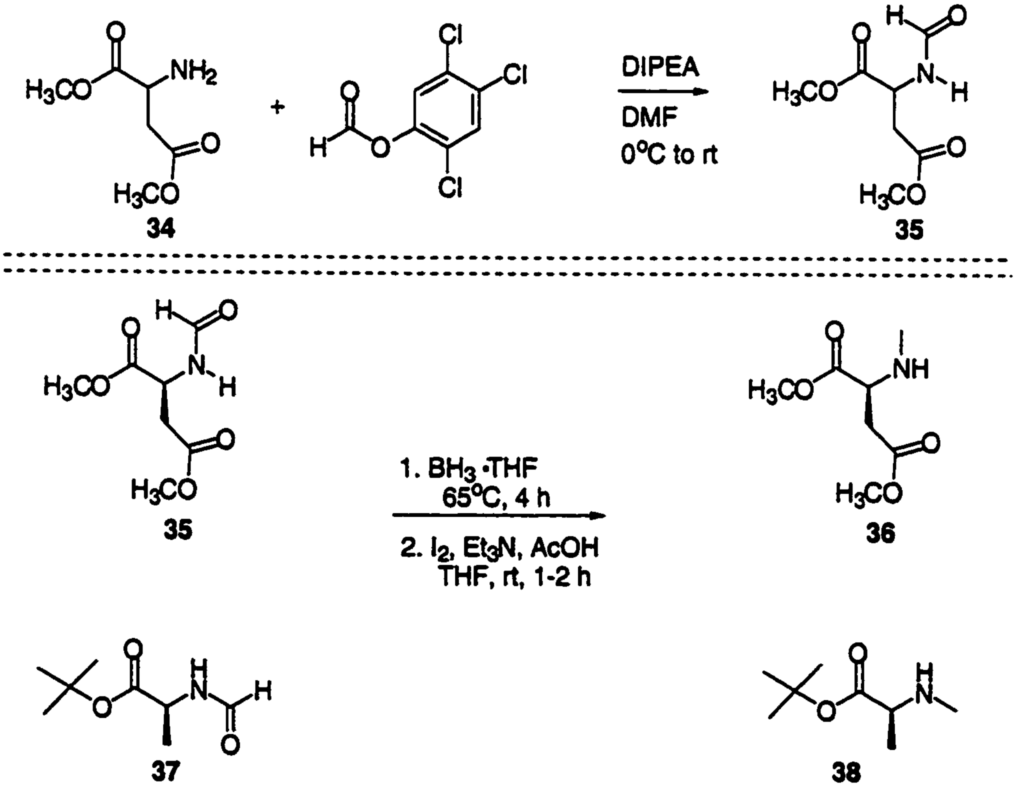
In summary, we hypothesize that the moniodoborane-amine adduct **27** is formed resulting in what would be a predicted stronger borane-amine adduct based on the accepted order of Lewis acidities.[25] As displacement of iodides in monoiodoborane amine adducts by charged nucleophiles is possible,[23] the acetoxyborane amine adduct **28** is formed next and subsequent facile breakdown of the adduct releases the free amine **29** to be trapped by the acetic acid.

Section 3: Solution Applications of the Borane Reduction/Iodine Work-up

Solution phase applications of the borane reduction/iodine work-up have been used to synthesize *N*-alkylated amino acid esters (Scheme 2.6). Preparation of the secondary amides from the free amines was straightforward except in the case of formylation of the dimethyl ester of glutamic acid **34** (Scheme 2.7). The original attempt at formylation was carried out using 2,4,5,-trichlorophenyl formate[26] and employed an aqueous work-up resulting in <10% yield. A second attempt (same conditions) without an aqueous work-up resulted in a 76% yield. Subsequent formylation reactions with other analogues avoided use of an aqueous work-up by concentrating the reaction mixture under reduced pressure and chromatographing the residue. Formation of the secondary amides with amino acid esters and propionic anhydride or acetic anhydride in the presence of triethylamine resulted in nearly quantitative yields.



Scheme 2.6: Synthesis of *N*-Alkylated Amino Acid Esters



Scheme 2.7: Formylated and Reduced Derivatives

In the borane reduction, the formylated dimethylester of glutamic acid **35**, and the *t*-butyl ester of alanine **37** analogues (**36** and **38** respectively) resulted in little to no yield. The low yields are presumably due to the aqueous work-up as the free amines exhibited high solubility in water and appeared to be volatile. In contrast, the reduction products **32** and **33** (Scheme 2.6) could be isolated and identified as crude mixtures with respectable yields of 71+%. The reduction of compound **30** required only 4 h of reflux for the reaction to go to completion resulting in a crude yield of 84%. The reduction of **31** however, after 4 h of reflux, resulted in a crude mixture of 1:1 starting material : product. It is suspected that steric factors reduce the rate of the reduction.

Section 4: Borane Reduction / Iodine Work-up of Peptides on Solid Support

In working towards the long term goal of synthesizing unnatural 'exo-peptides', attempts to construct several model 'natural' peptides on solid support were made as precursors for the borane reduction (Table 2.3). Unfortunately, all but one (entry 1) of the peptide construction attempts on high loading trityl resin (1% DVB) failed to render a clean crude peptide after TFA cleavage. The primary problem occurred at the coupling stage of the fifth residue resulting in add on and deletion sequences even with the use of longer coupling times (overnight), chaotropic salt (0.8M LiCl in DMF), and Triton X100. Difficult sequences are difficult to anticipate and eliminate and can occur at, and from the pentapeptide level.[27] Higher loading resins exacerbate the problem and difficult sequences are known to contain a high proportion of Ala, Val, and Ile.[28] Homoanalogues are known to have a tendency towards beta sheet formation, which is the suspected cause of resin shrinkage and lack of reagent penetration.[29]

Table 2.3: Peptide Construction Attempts on High Loading Resins

Entry #	Sequence on Solid Support[1]
1	Tr-hexadiamine-GlyAlaValGlyAlaVal-acetyl
2	Tr-hexadiamine-AlaPheLeuIleAla-acetyl[2]
3	Tr-hexadiamine-AlaPheValLeuNVal-acetyl[2]
4	Tr-m-xylylene diamine-AlaValNValPheLeu-acetyl
5	Tr-m-xylylene diamine-AlaValNValPheAla-acetyl
6	Tr-hexadiamine-AlaAlaAlaAlaAla-acetyl
7	Tr-hexadiamine-PhePhePhePhePhe-acetyl

1. Chlorotrityl resin, 1% DVB, 0.95 mmol/g loading from Novabiochem.
2. 2-Chlorotritylchloride resin, 1% DVB, 1.05 mmol/g loading from Novabiochem.

The peptide of entry 1 (Table 2.3) was constructed cleanly as shown by its resulting LRMS ES and ^{1}H NMR spectra, although three successive rounds of Fmoc deprotection and acetylation were required at the sixth residue to complete the sequence cleanly. Subjecting this resin bound peptide to 70 equivalents of borane for five days at 65°C and the iodine work-up resulted in near complete reduction (Table 2.4). Due to the oligoamine having an uninformative ^{1}H NMR spectrum (there are too many similar -NCH$_n$- signals), and total reduction being the goal, LRMS ES spectra was used as the initial indicator for complete or incomplete reduction. The predominant *m/z* peak of 515.5 indicated the desired product (M+1).

Table 2.4: Reduction Attempts

Entry #	Reaction Conditions[1]	Crude Yield %[2]	Major Peaks by ES-MS
1	70 equiv. 1 M BH_3•THF for 5 d at 65°C	100 +	515.5, 529.5, 537.5, 539.5, 551.5
2	Same as above + 70 equiv. each of boric acid and trimethyl borate	N / A	Recovered starting material only
3	10 M BH_3•THF + 140 equiv. in 2-methyl THF for 4 d	91	515.5, 527.6, 529.5, 539.6, 543.7, 553.6
4	140 equiv. 1 M BH_3•THF + 70 equiv. trimethyl borate in THF for 5 d at 65°C	100 +	515.6, 529.5, 539.5, 537.5
5	Same conditions as entry 1 and add another 70 equiv. of borane at 3 d into reaction.	100 +	515.6, 539.6, mess

1. All reduction reactions were performed on entry 1, Table 12. Reactions were performed on a 50–500 mg resin scale.
2. After TFA cleavage off of solid support.

A peak of M+14 indicated the incomplete reduction of one amide bond. We suspected that incomplete reduction during the reduction step was due to aggregation of the boron-amine adducts rendering further reagent penetration difficult. Houghten's reduction procedure for polyamides on solid support involves the use of trimethylborate, boric acid, and borane in large excess but equal molar amounts.[22] It has been reported that this mixture aids in the prevention of boron-amine aggregates[22,30] which is the suspected cause for incomplete reduction of polyamides on the solid support. Attempts at reduction of peptide 1 (entry 2) utilizing this protocol consistently failed to give any reduction product and only starting material was recovered. Upon addition of borane to the resin mixture containing boric acid, instantaneous and apparently more vigourous gas evolution was observed as compared to reduction attempts in the absence of boric acid. Recently, the same authors have reported their reduction protocol with 15 equivalents of trimethyl borate : 15 equivalents of boric acid: 40 equivalents of borane per amide bond[31] (previously stated 40 : 40 : 40). Also, the use of a higher boiling solvent (entry 3) was employed in an attempt to drive the reduction to completion, but this failed to render a better result. The use of trimethyl borate in the borane resulted in no difference in the ES-MS spectra profile to that of borane alone in the reductions.

By protecting the nitrogen-amide bond as a tertiary amide, prevention of aggregates (via N-B adduct formation) and a more facile reduction could result. Voyer's[32] method for silylation of carbamates was applied to peptide **1** on solid support. Single bead IR spectra indicated some O-alkylation. The solid support silylated peptide was subsequently reduced using the borane reduction/iodine reduction protocol and resulted in no improvement in the ES-MS reduction profile with considerably more peaks.

Preliminary acetylation attempts with acetic anhydride on solid support of the corresponding oligo(*N*-acylaziridine) of the peptide **1** precursor resulted in an insoluble material after trifluoroacetic acid cleavage. The material had some solubility in formic acid : methanol with sonification and subsequent identification of the desired product by ES-MS was possible although the spectrum was very poor.

III. CONCLUSION

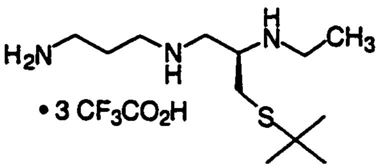
In conclusion, the borane reduction/iodine work-up is generally amenable to a wide variety of protected amino acid side chain functionality and has potential for a broad range of solution- and solid-phase applications. A hypothesis of the mechanistic pathway has been proposed illustrating the roles of all of the components in the buffered solution. Long chain 'exo-peptides' of six or more residues may exhibit solubility limitations. For a more facile synthesis and characterization of 'exo-peptides', future considerations should include the use of more polar side chain functionality, a polar 'tail', and the use of different resins with lower loading such as Tentagel and PS-PEG.

IV. EXPERIMENTAL PROCEDURES

General Methods: Same as stated in chapter one.

Typical synthesis of peptides on chlorotrityl resin with a diaminopropane linker. Chlorotrityl resin (1.07 mmol/g loading) was charged in ~ 4 equal portions (total of 1.09 g, 1.16 mmol), 15 min apart, with vortexing in between, at rt to a solution of 10 mL CH_2Cl_2 and 3 mL (40 + eq) of diaminopropane. The resin was vortexed for one hour after the addition of the last portion. Dry MeOH (3 mL) was charged to the mixture and vortexed for more 1 h at rt. The solution was drained and subsequently rinsed and vortexed with 3 × 10 mL CH_2Cl_2, 3 × 10 mL MeOH, 3 × CH_2Cl_2. The resin was dried under high vacuum for 12 h +. A solution of 4.0 eq HBTU (1.76 g), 4.0 eq of HOBt (0.71 g) and DIPEA (8.0 eq, 1.62 mL) in 5 mL DMF was charged to a mixture of the resin and 4.0 eq of Fmoc-Cys(*t*-Bu)-OH (1.85 g, 4.64 mmol) in 5 mL DMF. The resin mixture was vortexed for 3 h at rt and then the solution was drained from the resin. The resin was subsequently rinsed and vortexed with 3 × 10 mL DMF, 5 × 10 mL CH_2Cl_2. The resin was dried for 0.5 h under high vacuum and then tested for coupling completion by the ninhydrin assay. The resin was rinsed and vortexed with 3 × 5 mL DMF, 5 mL 4 : 1 DMF : piperidine solution (3 – 5 min), 2 × 5 mL DMF, and then 10 mL 4 : 1 DMF : piperidine solution for 30 + min at rt. The solution was drained and the resin was subsequently rinsed and vortexed with 5 × 10 mL DMF portions. A solution of ~30 eq (2.8 mL) of acetic anhydride and 6.0 eq (0.82 mL) of triethylamine in 10 mL of DMF was charged to the resin. After vortexing at rt for 3 – 4 h, the solution was drained from the resin. The resin was then rinsed and vortexed with 3 × 10 mL DMF, 3 × 10 mL MeOH and 3 × 10 mL CH_2Cl_2 portions. A weight of 1.02 g for the resin was obtained after drying under high vacuum for 12 h + (new loading of 0.96 mmol/g (based on original loading and mass balance)). The resin was subsequently subjected to the ninhydrin assay to test for coupling completion.

Typical preparation of triamines on solid support. A mixture of 24 mL dry THF and 24 mL of 1 M BH_3•THF was charged to a mixture of the cysteinyl peptide on trityl resin **16b** (0.46 g, 0.45 mmol) (prepared as mentioned above) in a presilanized round bottom flask, at rt. The mixture was heated to 65°C and was gently stirred for 5 d. After cooling to rt, the resin was transferred to a pp vessel with dry THF and subsequently rinsed with 3 × 5 mL THF portions. A solution of 10 mL THF, 2 mL of DIPEA, 4 mL of AcOH and 0.61 g of I_2 (4.0 eq) was charged to the resin and the resulting mixture was vortexed for 3 – 4 h at rt. The solution was drained and the resin was subsequently rinsed with 3 × 5 mL THF, 3 × 5 mL 4 : 1 DMF : triethylamine, 3 × 5 mL MeOH and 3 × 5 mL CH_2Cl_2 portions. The resin was dried under high vacuum for 12 h and 0.62 g resin was obtained (new loading of 0.98 mmol/g).

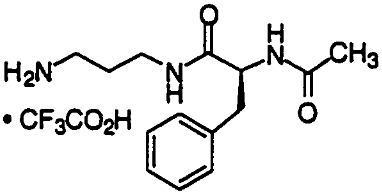


Compound 19b

Typical cleavage of peptides/triamines off solid support:

(4*R*)-(*t*-Butyl)thiomethyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (19b). Dry CH_2Cl_2 (4.75 mL) was charged to 0.46 g (0.45 mmol) of the prepared resin bound cysteinyl triamine **18b** followed by 50 μL of ethanedithiol (100 μL triisopropylsilane was used instead if the peptide or triamine did not contain sulfur) and 0.25 mL TFA. The resin was slurried for 0.5 h at rt and then filtered and rinsed through a glass frit filter into a fitted round bottom flask with 3 × 10 mL CH_2Cl_2 and 10 mL 1 : 1 CH_2Cl_2 : dry MeOH. The filtrate was concentrated under reduced pressure in a bath that was below 20°C. Ether (20 mL) was slowly added to the resulting residue with vigorous swirling of the flask. The ether was then decanted and the remaining residue was placed under high vacuum for 12 h to result in 0.20 g of a pale yellow oil for a crude yield of 79%. $[\alpha]^{25}_{D} = + 6.7°$ (c = 11.9, MeOH); IR (MeOH cast) 3412, 2968, 1684, 1464, 1432, 1369, 1208, 1026, 838, 800, 761, 723, 598, 518, 443, and 412 cm^{-1}; ^{1}H NMR (CD_3OD) δ 5.05 (br s, NH), 3.77 (m, 1 H, -CHN-), 3.49 (d, *J* = 5.5, 2 H, $-NCH_2CHN-$), 3.34 – 2.93 (m, 7 H, $-CH_2S-$, $-NCH_2-$), 2.97 (dd, *J* = 13.7, 7.1, 1 H, $-CH_2S-$), 2.13 (m, 2 H, $-CH_2CH_2CH_2-$), 1.35 (t, *J* = 7.2, 3 H, CH_3), 1.35 (s, 1.35, 9 H, *t*-Bu); ^{13}C NMR (CD_3OD) δ 163.1 (q, *J* = 0.5, CO of TFA), 118.0 (q, *J* = 3.9, CF_3 of TFA), 56.4 (-

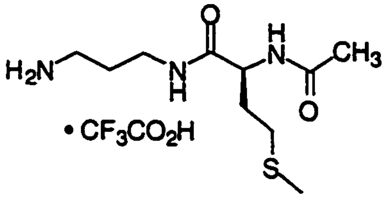
CHN-), 48.9 (-CH_2N-), 46.9 (-CH_2N-), 44.7 (-CH_2N-), 42.1 (-CH_2N-), 37.7 (-CH_2S-), 31.0 (CH_3), 28.1 (-$SC(CH_3)_3$), 25.2 (-CH_2**CH_2**CH_2-), 11.5 (CH_3); HRMS ES in MeOH for $C_{12}H_{30}N_3S$ (M^++ H) (calcd 248.216045) found 248.215786.



Compound 17g

1-(3-aminopropanyl)-*N*-α-acetyl-(L)-phenylalanylamide trifluoroacetatic acid salt (17g).

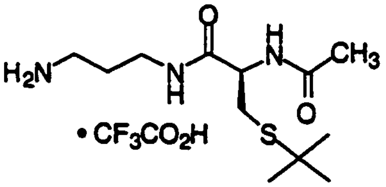
Peptide **16g** was constructed on solid support as above. A quantity of resin (0.25 g, 0.23 mmol) was cleaved as stated above to give 80.6 mg of an off-white solid for a crude yield of 98%. $[\alpha]^{25}_D$ = + 11.9° (c = 8.1, MeOH, unstable reading); IR (MeOH cast) 3278, 3065, 1674, 1540, 1455, 1437, 1375, 1285, 1204, 1135, 1032, 837, 800, 748, 722, 701, 597, 517 cm^{-1}; ^1H NMR (CD_3OD) δ 7.25 (m, 5 H, H-Ph), 4.47 (apparent t, *J* = 7.6, 1 H, -CHN-), 3.20 (m, 2 H, -CH_2N-), 3.06 (dd, *J* = 13.7, 6.9, 1 H, -CH_2Ph), 2.90 (dd, *J* = 13.7, 8.4, 1 H, -CH_2Ph), 2.79 (m, 2 H, -CH_2N-), 1.90 (s, 3 H, CH_3), 1.74 (m, 2 H, -CH_2**CH_2**CH_2-); LRMS ES in MeOH for $C_{14}H_{21}N_3O_2$ found 236.2 (M^++ H).



Compound 17e

1-(3-aminopropanyl)-*N*-α-acetyl-(L)-methionylamide trifluoroacetatic acid salt (17e).

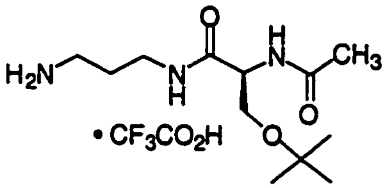
Peptide **16e** was constructed on solid support as above. A quantity of resin (0.16 g, 0.15 mmol) was cleaved as stated above to give 38.6 mg of an oily white solid (73% crude yield). $[\alpha]^{25}_{D}$ = - 13.1° (c = 1.9, MeOH, turbid solution); IR (MeOH cast) 3276, 3065, 1675, 1537, 1434, 1375, 1291, 1203, 1134, 836, 800, 722 cm^{-1}; ^{1}H NMR (CD$_3$OD) δ 4.32 (dd, *J* = 5.3, 5.2, 1 H, -NCH), 3.30 (m, 2 H, - CH$_2$CH$_2$S-), 2.93 (t, *J* = 7.3, 2 H, -CH$_2$N-), 2.51 (m, 2 H, -CH$_2$N-), 2.10 (s, 3 H, CH$_3$), 2.00 (s, 3 H, CH$_3$), 1.93 (m, 2 H, -CH$_2$CH$_2$CH$_2$-), 1.84 (t, *J* = 7.1, 2 H, -CH$_2$S-); LRMS ES in MeOH for C$_{10}$H$_{21}$N$_3$O$_2$S found 248.1 (M$^+$+ H).



Compound 17b

1-(3-aminopropanyl)-*N*-α-acetyl-S-*t*-butyl-(L)-cysteinylamide trifluoroacetatic acid salt (17b).

Peptide **16b** was constructed on solid support as above. A quantity of resin (0.11 g, 0.11 mmol) was cleaved as stated above to give 40.4 mg of an oily white solid for a crude yield of 94%. $[\alpha]^{25}_{D}$ = - 8.0° (c = 4.6, MeOH); IR (MeOH cast) 341, 3081, 2965, 1675, 1540, 1463, 1435, 1368, 1204, 1138, 838, 801, 723, 597 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.36 (t, J = 7.1, 1 H, -CHN-), 3.30 (m, 2 H, $-CH_2N-$), 2.96 (t, J = 7.2, 2 H, $-CH_2N-$), 2.94 (dd, J = 12.7, 6.8, 1 H, $-CH_2S-$), 2.81 (dd, J = 12.7, 7.4, 1 H, $-CH_2S-$), 1.98 (s, 3 H, $-CH_3$), 1.85 (m, 2 H, $-CH_2\mathbf{CH_2}CH_2-$), 1.32 (s, 9 H, *t*-Bu); LRMS ES in MeOH for $C_{12}H_{25}N_3O_3S$ found 276.1 (M^{+}+ H).

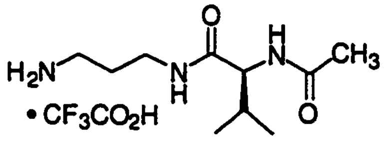


Compound 17h

1-(3-aminopropanyl)-*N*-α-acetyl-O-*t*-butyl-(L)-serylamide trifluoroacetatic acid salt (17h).

Peptide **16h** was constructed on solid support as above. A quantity of resin (0.22 g, 0.18 mmol) was cleaved as stated above to give 69 mg of an off-white solid (quantitative crude yield). : $[\alpha]^{25}_{D}$ = - 2.1° (c = 6.9, MeOH); IR (MeOH cast) 3282, 2976, 1675, 1538, 1435, 1393, 1366, 1259, 1202, 1135, 835, 799, and 722 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.33 (apparent t, J = 5.1, 1 H, CHN), 3.67 (dd, J = 9.2, 4.9, 1 H, $-CH_2O-$), 3.56 (dd, J = 9.2, 5.3, 1 H, $-CH_2O-$), 3.31 (t, J = 6.4, 2 H, $-CH_2N-$), 2.95 (

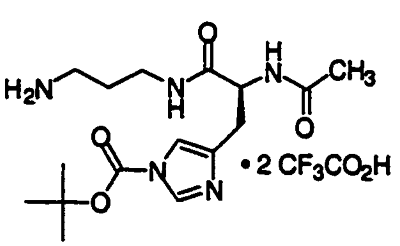
t, J = 7.1, 2 H, -CH_2N-), 2.01 (s, 3 H, -CH_3), 1.84 (m, 2 H, -$CH_2CH_2CH_2$-), 1.17 (s, 9 H, *t*-Bu) ; LRMS ES in MeOH for $C_{12}H_{25}N_3O_3$ (M + 1), found 260.2 (M^++ H).



Compound 17a

1-(3-aminopropanyl)-*N*-α-acetyl-(L)-valylamide trifluoroacetatic acid salt (17a).

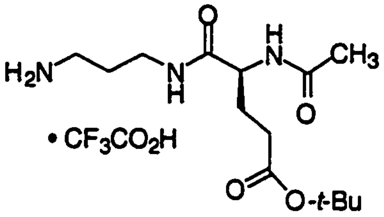
Tripeptide **16a** was constructed on solid support as above. A quantity of resin (0.25 g, 0.19 mmol) was cleaved as stated above to give 60.7 mg of an oily off-white solid for a crude yield of 97%. $[\alpha]^{25}_D$ = - 13.9° (c = 4.6, in MeOH); IR (neat) 3283, 2967, 1655, 1543, 1469, 1434, 1375, 1203, 1181, 1136, 836, 800, 722 cm^{-1}; ^1H NMR (CD_3OD) δ 4.01 (d, J = 7.2, 1 H, α-CH), 3.30 (m, 2 H, -CH_2N-), 2.94 (t, J = 7.3, 2 H, -CH_2N-), 2.05 (m, 1 H, CH), 1.99 (s, 3 H, CH_3), 1.84 (m, 2 H, -$CH_2CH_2CH_2$-), 0.96 (d, J= 6.8, 3 H, CH_3), 0.95 (d, J = 6.8, 3 H, CH_3): LRMS ES in MeOH for $C_{10}H_{21}N_3O_2$ found 216.1 (M^++ H).



Compound 17d

1-(3-aminopropanyl)-*N*-α-acetyl-*N*-im-*t*-butoxycarbonyl-(L)-histidylamide bis(trifluoroacetatic) acid salt (17d).

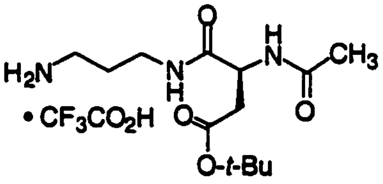
Peptide **16d** was constructed on solid support as before except for conversion of the amino acid to its free acid form before the coupling step. To a suspension of *N*-α-Fmoc-*N*-im-*t*-Boc-(L)-histidine cyclohexylammonium salt (2.39 g, 4.14 mmol) in 20 mL EtOAc, 2.5 mL of 2 M H_2SO_4 (1.2 eq) is charged and the mixture is shaken until all solids are dissolved. The layers are separated. The aqueous layer is charged with 10 mL of cold H_2O and is extracted twice with 20 mL EtOAc. The organic layers are combined, washed with 2 × 20 mL of H_2O, dried with $MgSO_4$, and concentrated in a rotary evaporator at <40°C. The residue is placed under high vacuum for 1 h and then used immediately for coupling and the peptide is constructed as stated above. A quantity of resin (0.22 g, 0.18 mmol) is cleaved as stated above to give 67.9 mg of an off white solid for a crude yield of 66%. $[\alpha]^{25}_D$ = - 2.8° (c = 5.6, MeOH); IR (MeOH cast) 3032, 1674, 1538, 1432, 1376, 1203, 1132, 835, 799, 722, 629, 517 cm^{-1}; ^1H NMR (CD_3OD) δ 8.78 (s, 1 H , ring CH), 7.30 (s, 1 H, ring CH), 4.90 (br s, NH) 4.62 (dd, *J* = 8.88, 5.49, 1 H, CH), 3.3 (m, 3 H, -CH**CH_2**-, -**CH_2**N-), 3.09 (dd, *J* = 14.38, 8.98, 1 H, -CH**CH_2**-), 2.93 (t, *J* = 7.3, 2 H, -CH_2N-), 1.95 (s, 3 H, CH_3), 1.84 (m, 2 H, -CH_2**CH_2**CH_2-), 1.55 (s, 2 H, *t*-Bu (protecting group is partially removed)). LRMS ES in MeOH for $C_{16}H_{25}N_5O_4$ found 312.1 (M^++ H).



Compound 17c

1-(3-aminopropanyl)-*N*-α-acetyl-O-*t*-butyl-(L)-glutamylamide trifluoroacetatic acid salt (17c).

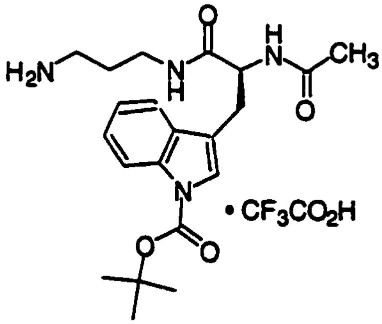
Peptide **16c** was constructed on solid support as stated above. A quantity of resin (0.28 mg, 0.23 mmol) was cleaved as above to give 88.2 mg of a clear colorless oil for a crude yield of 92%. $[\alpha]^{25}_{D}$ = - 6.7° (c = 6.3, MeOH); IR (MeOH cast) 3277, 2980, 1674, 1538, 1435, 1370, 1256, 1202, 1178, 1156, 1134, 836, 799, 722, 418 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.2 (dd, J = 8.9, 5.4, 1 H, α-CH), 3.3 (m, 2 H, -COCH$_2$CH$_2$-), 2.93 (t, J = 7.2, -CH$_2$N-), 2.31 (t, 2 H, J = 7.4, -CH$_2$N-), 2.05 (m, 2 H, -CHCH$_2$-), 1.97 (s, 3 H, CH$_3$), 1.83 (m, 2 H, -CH$_2$CH$_2$CH$_2$-), 1.42 (s, 9 H, *t*-Bu); LRMS ES in MeOH for $C_{14}H_{27}N_3O_4$ found 302.3 (M^++ H).



Compound 17j

1-(3-aminopropanyl)-*N*-α-acetyl-O-*t*-butyl-(L)-aspartylamide trifluoroacetatic acid salt (17i).

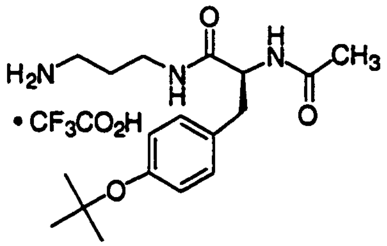
Peptide **16i** was constructed on solid support as above. A quantity of resin (0.27 mg, 0.23 mmol) was cleaved to give 59.0 mg of an oily off white solid for a crude yield of 89%. $[\alpha]^{25}_{D} = - 12.8°$ (c = 5.9, MeOH); IR (MeOH cast) 3852, 3815, 3743, 3064, 2925, 1958, 1652, 1575, 1561, 1538, 1505, 1455, 1384, 1202, 1142, 1073, 838, 800, 722, 700, 472 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.62 (dd, J = 7.8, 5.9, 1 H, -CH-), 3.30 (m, 2 H, $-CH_2N-$), 2.94 (t, J = 7.2, 2 H, $-CH_2N-$), 2.76 (dd, J = 16.3, 5.9, 1 H, $-CHCH_2-$), 2.60 (dd, J = 16.3, 7.8, 1 H, $-CHCH_2-$), 1.98 (s, 3 H, CH_3), 1.84 (m, $-CH_2CH_2CH_2-$), 1.4 (s, 9 H , *t*-Bu); LRMS ES in MeOH for $C_{13}H_{25}N_3O_4$ found 288.3 (M^{+}+ H).



Compound 17j

1-(3-aminopropanyl)-*N*-α-acetyl-*N*-in-*t*-butoxycarbonyl-(L)-tryptophylamide trifluoroacetatic acid salt (17j).

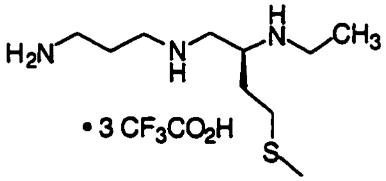
Peptide **16j** was constructed on solid support as above. A quantity of resin (0.15 g, 0.13 mmol) was cleaved as stated above to give 50.8 mg of an oily white solid for a crude yield of 76%. $[\alpha]^{25}_{D}$ = (+) 13.1° (c = 4.6, MeOH, turbid solution); IR (MeOH cast) 3277, 2941, 1733, 1674, 1652, 1538, 14563, 1371, 1330, 1309, 1257, 1203, 1158, 1087, 1018, 836, 800, 767, 747, 722 cm^{-1}; ^1H NMR (CD_3OD) δ 8.06 (d, *J* = 8.1, 1 H, ring H), 7.58 (d, *J* = 8.2, 1 H, ring H), 7.50 - 6.96 (m, 3 H, ring H), 4.47 (dd, *J* = 8.3, 8.2, 1 H, CH), 3.28 - 3.06 (m, 3 H, -CH_2-indole, -CH_2N-), 3.02 (dd, *J* = 14.8, 8.3, 1 H, -CH_2-indole), 2.84 (t, *J* = 7.1, 2 H, CH_2N), 1.93 (s, 3 H, CH_3), 1.75 (m, 2 H, -$CH_2CH_2CH_2$-), 1.65 (s, 9 H, *t*-Bu); LRMS ES in MeOH for $C_{21}H_{30}N_4O_4$ found 403.3 (M^+ + H).



Compound 17f

1-(3-aminopropanyl)-*N*-α-acetyl-O-*t*-butyl-(L)-tyrosylamide trifluoroacetatic acid salt (17f).

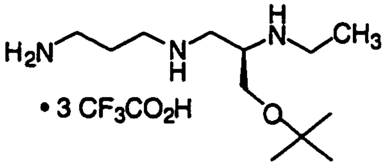
Peptide **16f** was constructed on solid support as above. A quantity of resin (30.1 mg, 0.029 mmol) was cleaved to give 11.7 mg of an oily white solid for a crude yield of 89%. $[\alpha]^{25}_{D}$ = + 20.9° (c = 0.9, MeOH); IR (MeOH cast) 3278, 2932, 1673, 1652, 1538, 1507, 1439, 1368, 1238, 1203, 1180, 1135, 1051, 896, 836, 800, 722, 703, 418 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.13 (d, *J* = 8.5, 2 H, ring CH), 6.91 (d, *J* = 8.4, 2 H, ring CH), 4.40 (apparent t, *J* = 7.6, 1 H, -CHN-), 3.15 (m, 2 H, -CH_2N-), 3.05 (dd, *J* = 13.6, 7.1, 1 H, -**CH_2**Ph), 2.87 (dd, *J* = 13.6, 8.2, 1 H, -**CH_2**Ph), 2.81 (m, 2 H, -CH_2N-), 1.91 (s, 3 H, CH_3). 1.74 (m, 2 H, -CH_2**CH_2**CH_2-), 1.30 (s, 9 H, *t*-Bu); LRMS ES in MeOH for $C_{18}H_{24}N_3O_3$ found 336.2 (M^++ H).



Compound 19e

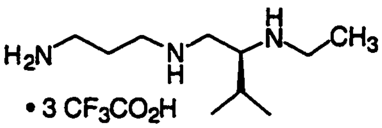
(4*S*)-(2'-Methylthio)ethyl-9-amino-3,6-diazononane tris(trifluoroacetatic acid) salt (19e). The triamine **18e** was constructed on solid support as mentioned above. Cleavage of the triamine off of the resin (0.48 g, 0.46 mmol) was as before except there was no MeOH rinse forward. A pale yellow oil (0.20 g) was obtained to give a 78% crude yield. $[\alpha]^{25}_D$ = + 7.7° (c = 5.9, MeOH, turbid solution); IR (MeOH cast) 2999, 1675, 1431, 1202, 1133, 837, 799, 722 cm^{-1}; ^{1}H NMR (CD_3OD) δ 4.90 (br s, NH), 3.71 (m, 1 H, -CHN-), 3.43 (d, *J* = 5.3, 2 H, -NCH_2-), 3.31 – 3.10 (m, 4 H, -NCH_2-), 3.06 (t, *J* = 7.6, 2 H, -NCH_2-), 2.65 (m, 2 H, -CH_2S-), 2.12 (s, 3 H, -SCH_3), 2.16-1.98 (m, 4 H, -CH_2**CH_2**CH_2-, -**CH_2**CH_2S-), 1.34 (t, *J* = 7.1, 3 H, -CH_3); ^{13}C NMR (CD_3OD) δ163.0 (q, *J* = 0.5, CO of TFA), 118.0 (q, *J* = 3.9, CF_3 of TFA), 55.8 (-CHN-), 48.8 (-CH_2N-), 46.9 (-CH_2N-), 42.1 (-CH_2N-), 37.8 (-CH_2N-), 30.0 (-

CH_2N-), 29.3 (-SCH_3 or –CH_2S-), 25.3 (-SCH_3 or –CH_2S-), 15.1 (-CH_2**CH_2**CH_2-), 11.6 (CH_3); HRMS ES in MeOH for $C_{10}H_{26}N_3S$ (M^++ H) (calcd 220.184745) found 220.185011.



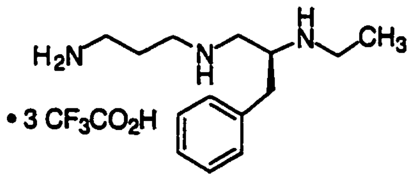
Compound 19h

(4*R*)-*t*-Butoxymethyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (19h). Triamine **18h** was constructed on solid support as above. Cleavage of the triamine off of the resin (0.48 g, 0.39 mmol) was as mentioned above except there was no MeOH rinse forward. A white oily solid (0.14 g) was obtained to give a 61% crude yield. $[\alpha]^{25}_{D}$ = - 2.2° (c = 2.5, MeOH); IR (MeOH cast) 2981, 1675, 1431, 1369, 1203, 1135, 837, 799, 722, 598, 517cm^{-1}; ^{1}H NMR (CD_3OD) δ 3.80 (m, 3 H, -CHN-, -CH_2O-), 3.49 (d, *J* = 4.7, 2 H, -CH_2N-), 3.40 (m, 4 H, -CH_2N-), 3.07 (t, *J* = 7.6, 2 H, -CH_2N-), 2.15 (m, 2 H, -CH_2**CH_2**CH_2-), 1.35 (t, *J* = 7.2, 3 H, CH_3), 1.23 (s, 9 H, *t*-Bu); ^{13}C NMR (CD_3OD) δ 163.1 (q, *J* = 0.5, CO of TFA), 118.0 (q, *J* = 3.9, CF_3 of TFA), 76.0 (-CH_2O-), 59.4 (-$OC(CH_3)_3$), 55.7 (-CHN-), 47.7 (-CH_2N-), 46.9 (-CH_2N-), 42.2 (-CH_2N-), 37.7 (-CH_2N-), 27.4 (C(**CH_3**)$_3$), 25.3 (-CH_2**CH_2**CH_2-), 11.4 (CH_3); HRMS ES in MeOH for $C_{12}H_{30}N_3O$ (M^++ H) (calcd 232.238888) found 232.238733.



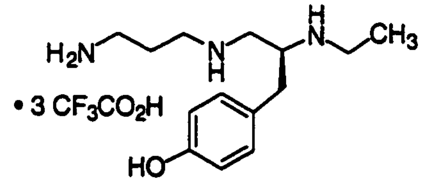
Compound 19a

(4*R*)-Isopropyl-9-amino-3,6-diazononane tris(trifluoroacetatic acid) salt (19a). Triamine **18a** was constructed on solid support as above. Cleavage of the triamine off of the resin (0.38 g / 0.37 mmol) was as mentioned above. An oily white solid (0.16 g) was obtained to give a 79 % crude yield. $[\alpha]^{25}_{D} = + 10.0°$ (c = 2.4, MeOH); IR (neat) 2978, 1676, 1469, 1430, 1203, 1134, 837, 799, 722, 598, 518; ^{1}H NMR (CD_3OD) δ 3.47 (m, 1 H, α-CH), 3.41 (d, *J* = 3.5, 2 H, -NCH_2-), 3.31 – 3.17 (m, 4 H, -CH_2N-), 3.07 (t, *J* = 7.7, -CH_2N-), 2.28 (m, 1 H, -CH-), 2.14 (m, 2 H, -CH_2**CH_2**CH_2-), 1.36 (t, *J* = 7.2, 3 H, -CH_2**CH_3**), 1.10 (d, *J* = 6.6, 3 H, CH_3), 1.05 (d, *J* = 7.0, 3 H, CH_3); ^{13}C NMR (CD_3OD) δ 61.7 (CHN), 47.0 (-CH_2N-), 46.9 (-CH_2N-), 43.1 (-CH_2N-), 37.8 (-CH_2N-), 28.7 (-CH**CH**$(CH_3)_2$), 25.5 (-CH_2**CH_2**CH_2-), 18.3 (CH_3), 16.6 (CH_3), 11.4 (CH_3); HRMS ES in MeOH for $C_{10}H_{26}N_3$ (M^++ H) (calcd 188.212673) found 188.212895.



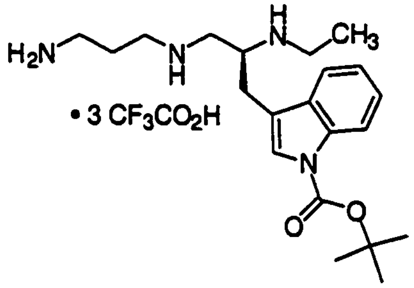
Compound 19g

(4*S*)-Benzyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (19g). Triamine **18g** was constructed on solid support as above. Cleavage of the triamine off of the resin (0.44 g, 0.41 mmol) was as mentioned above. An off-white solid (0.18 g) was obtained to give a crude yield of 75 %. $[\alpha]^{25}_D$ = + 8.5° (c = 6.2, MeOH); IR (MeOH cast) 3031, 1683, 1498, 1457, 1431, 1204, 1135, 1031, 838, 799, 748, 723, 703, 599, 518, 442, 411 cm^{-1}; ^{1}H NMR (CD_3OD) δ 7.5 (m, 5 H, H-Ph), 3.93 (m, 1 H, -CHN-), 3.51 (dd, *J* = 14.2, 7.7, 1 H, -CH_2Ph-), 3.30 – 2.95 (m, 5 H, -CH_2Ph, -CH_2N-), 3.11 (t, *J* = 7.6, 2 H, -CH_2N-), 3.03 (t, *J* = 7.7, 2 H, -CH_2N-), 2.08 (m, 2 H, -CH_2**CH_2**CH_2-), 1.32 (t, *J* = 7.2, 3 H, -CH_3); ^{13}C NMR (CD_3OD) δ 163.2 (q, *J* = 0.5, CO of TFA), 135.9 (C of ring), 130.4 (CH of ring), 130.3 (CH of ring), 128.9 (CH of ring), 57.9 (-CHN-), 48.9 (-CH_2N-), 46.8 (-CH_2N-), 42.3 (-CH_2N-), 37.7 (-CH_2N-), 36.0 (-**CH_2**Ph), 25.4 (-CH_2**CH_2**CH_2-), 11.6 (CH_3); HRMS ES in MeOH for $C_{14}H_{26}N_3$ (M + 1) (calcd 236.212673) found 236.212665.



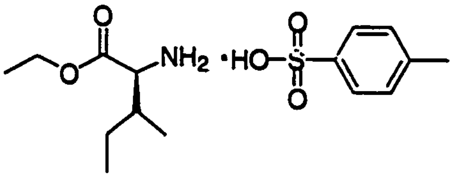
Compound 19f (-*t*-Bu)

(4*S*)-*p*-Hydroxybenzyl-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (19f (-*t*-Bu)). Triamine **18f** was constructed on solid support as above. Cleavage of the triamine off of the resin (0.40 g, 0.39 mmol) was as mentioned above. In order to characterize this compound in its deprotected form, the resulting concentrated crude residue (0.175 g) was then treated with a 95 : 5 : 5 TFA : triisopropylsilane: H_2O cleavage cocktail for 4 h at rt. The mixture was concentrated under reduced pressure and 20 mL of Et_2O was added slowly to the residue. After precipitation, the Et_2O was decanted off and the residue was placed under high vacuum for 12 h to give 0.15 g of an off-white solid (66% overall crude yield). $[\alpha]^{25}_D = + 6.6°$ (c = 6.4, MeOH, erratic reading); IR (MeOH cast) 3399, 3011, 1678, 1614, 1519, 1456, 1436, 1202, 1133, 838, 799, 722 cm^{-1}; ^1H NMR (CD_3OD) δ 7.13 (d, *J* = 8.4, 2 H, ring H), 6.78 (d, *J* = 8.4, 2 H, ring H), 5.00 (br s, NH or OH)), 3.84 (m, 1 H, -CHN-), 3.47 (m, 1 H, -CH_2Ph), 3.30-3.05 (m, 5 H, -CH_2Ph, -NCH_2-), 3.01 (t, *J* = 7.6, 3 H, -NCH_2-), 2.85 (dd, *J* = 14.4, 8.7, 1 H, -CH_2Ph), 2.60 (m, 2 H, -CH_2**CH_2**CH_2-), 1.30 (t, *J* = 7.2, 3 H, CH_3); ^{13}C NMR (CD_3OD) δ 158.3 (HOC of ring), 131.5 (CH of ring), 126.1 (CH of ring), 117.1 (C of ring), 58.0 (-CHN-), 48.9 (-CH_2N-), 46.8 (-CH_2N-), 42.3 (-CH_2N-), 37.7 (-CH_2N-), 35.2 (-**CH_2**Ph), 25.3 (-CH_2**CH_2**CH_2-), 11.6 (CH_3); HRMS-ES in MeOH for $C_{14}H_{20}N_3O$ (M + 1) (calcd 252.207588) found 252.207932.



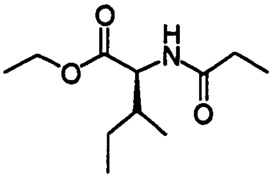
Compound 19j

(4*R*)-(3-Methyl-*N*-in-*t*-butoxyindolyl)-9-amino-3,6-diazanonane tris(trifluoroacetatic acid) salt (19j). Triamine **18j** was constructed on solid support as above. Cleavage of the triamine off of the resin (0.46 g, 0.32 mmol) was as before except there was no MeOH rinse forward. A light brown solid (0.18 g) was obtained to give a 79% crude yield. $[\alpha]^{25}_{D} = +1.6°$ (c = 6.6, MeOH, erratic reading); IR (MeOH cast) 3428, 2984, 1678, 1454, 1431, 1372, 1310, 1259, 1204, 1093, 837, 800, 768, 750, 723 cm^{-1}; ^{1}H NMR (CD_3OD) δ 8.14 (d, *J* = 8.1, 1 H, indole), 7.71 (s, 1 H, indole), 7.64 (d, *J* = 7.3, 2 H, indole), 7.30 (m, 2 H, indole), 5.00 (br s), 4.00 (m, 1 H, -CHN-), 3.50 (dd, *J* = 14.1, 7.7, 1 H, -$\mathbf{CH_2}$-indole), 3.40 to 2.95 (m, 9 H, -CH_2N-, -$\mathbf{CH_2}$-indole), 2.10 (m, 2 H, -$CH_2\mathbf{CH_2}CH_2$-), 1.66 (s, 9 H, *t*-Bu), 1.32 (t, *J* = 7.1, 3 H, CH_3); ^{13}C NMR (CD_3OD) δ 163.2 (q, *J* = 0.4, CO of TFA), 150.7 (CO of Boc), 137.0 (C of indole), 130.8 (C of indole), 126.5 (CH of indole), 125.0 (CH of indole), 124.1 (CH of indole), 119.8 (CH of indole), 114.5 (C of indole), 86.3 (-OC(CH_3)$_3$), 56.4 (CHN), 49.2 (-CH_2N-) 46.9 (-CH_2N-), 42.0 (-CH_2N-), 37.8 (-CH_2N-), 28.3 (-OC($\mathbf{CH_3}$)$_3$), 25.9 (-$CH_2\mathbf{CH_2}CH_2$- or CH_2-indole), 25.5 (-$CH_2\mathbf{CH_2}CH_2$- or CH_2-indole), 11.6 (CH_3); HRMS ES in MeOH for $C_{21}H_{35}N_4O_2$ (M^++ H) (calcd 375.276002) found 375.276561.



Compound 30a

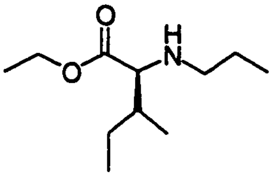
(L)-isoleucine ethyl ester p-tolylsulfonate salt (30a) . L-Isoleucine (4.01 g, 30.6 mmol) and p-toluene sulfonic acid (11.62 g, 61.1 mmol) were charged to 150 mL of absolute ethanol at rt and the resulting solution was refluxed for 20 h in the presence of a Dean Stark apparatus. The reaction mixture was concentrated under reduced pressure and the resulting white solid was slurried with 100 mL Et_2O. The slurry mixture was filtered and rinsed forward with 100 mL Et_2O. The isolated solid was dried under high vacuum for ~1.5 h and 5.6 g (52% yield) of a white solid was obtained. ^1H NMR ($CDCl_3$) δ 8.10 (br s, 2 H, NH), 7.73 (d, *J* = 8.2, 2 H, ring H), 7.13 (d, *J* = 8.5, 2 H, ring H), 4.10 (m, 2 H, OCH_2CH_3), 3.95 (m, 1 H, α-CH), 2.35 (s, 3 H, ringCH_3), 1.95 (m, 1H, *β*-CH), 1.45-1.15 (m, 2 H, -$CHCH_2CH_3$), 1.18 (t, *J* = 7.1, 3 H, -OCH_2**CH_3**), 0.78 (d, *J* = 7.0, 3 H, -CH**CH_3**). 0.55 (t, *J* = 7.3, 3 H, -$CHCH_2$**CH_3**).



Compound 30

***N*-α-propionyl-(L)-isoleucine ethyl ester (30).** Propionic anhydride (7.8 mL, 61.2 mmol) was charged to a solution of p-tolylsulfonate salt of L- isoleucine ethyl ester (4.28 g, 12.2 mmol) and triethylamine (3.4 mL, 24.5 mmol) in 50 mL THF and was left to stir at rt for 20 h. The resulting solution was concentrated under reduced pressure. The residue was purified by flash chromatography (4:1 Hexanes: EtOAc) and 2.61 g (98% yield) of a colorless oil was obtained. ^1H NMR ($CDCl_3$) δ 5.95 (br s, 1 H, NH), 4.59 (apparent dd, *J* = 8.6, 8.6, 1 H, α-CH), 4.17 (m, 2 H, -O**CH_2**CH_3), 2.25 (q, *J* = 7.6, 2 H, (CO)**CH_2**-), 1.86 (m, 1 H, *β*-CH), 1.45 (m, 1 H, -CH**CH_2**CH_3)

1.26 (t, *J* = 7.2, 3 H, -OCH_2CH_3), 1.16 (m, 1 H, -$CHCH_2CH_3$), 1.15 (t, *J* = 7.5, 3 H, CH_3), 0.90 (m, 6 H, CH_3).



Compound 32

***N*-propyl-(L)-isoleucine ethyl ester (32).** BH_3 in THF (14.5 mL, 14.5 mmol) was charged dropwise over 5 min to a solution of amido ester (compound **30**, 1.26 g, 5.81 mmol)) and 6.7 mL dry THF at 0°C. The reaction mixture was brought to reflux for 17 h. The reaction mixture was cooled to rt and triethylamine (2 mL), glacial AcOH (3 mL) and of I_2 (1.78 g, 7 mmol) were successively added. The solution was stirred for 1 h at rt. Contents were then transferred to a separatory funnel with 100 mL 5% NaOH solution. The pH was adjusted to pH 11+ with solid NaOH pellets and then aq. sat. $Na_2S_2O_3$ (10 mL) was added. The solution was extracted with Et_2O (3 × 5 mL), combined and washed with brine and dried over anhydrous $MgSO_4$. The resulting solution was filtered, concentrated under reduced pressure and placed on high vacuum for 1 h. A crude brown oil 0.78 g (82% yield) was obtained. ^{1}H NMR ($CDCl_3$) δ 4.13 (m, 2 H, CH_3**CH_2**O-), 2.98 (d, *J* = 7.0, 1 H, -CHN-), 2.59-2.29 (m, 2 H, -NCH_2-), 1.65-1.30 (m, 3 H, *β*-CH, -NCH_2**CH_2**-), 1.10 (m, 2 H, -CH**CH_2**CH_3), 0.85 (m, 12 H, CH_3); ^{13}C NMR ($CDCl_3$) δ 175.2 (CO), 66.1 (CHN), 60.3 (CH_3**CH_2**O-), 50.6 (-NCH_2-), 38.4 (CH), 25.9 (-CH_2-), 23.3 (-CH_2-), 15.5 (CH_3), 14.4 (CH_3), 11.7 (CH_3), 11.5 (CH_3); $[\alpha]^{25}_{D} = + 3.7°$ (c = 14.2, $CHCl_3$); HRMS EI calculated for $C_{11}H_{23}O_2N$ (201.7288) found 201.7211.

V. NOTES AND REFERENCES

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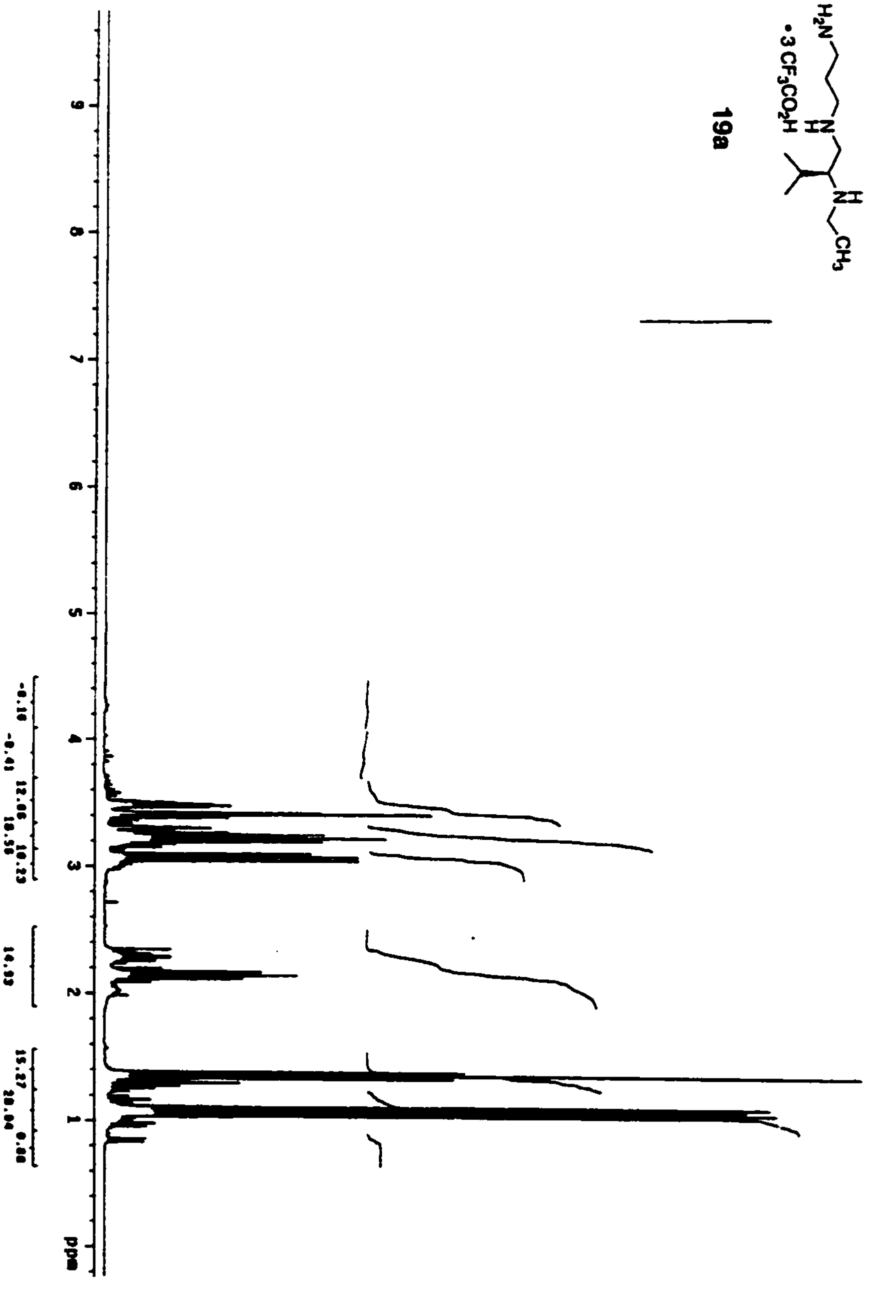
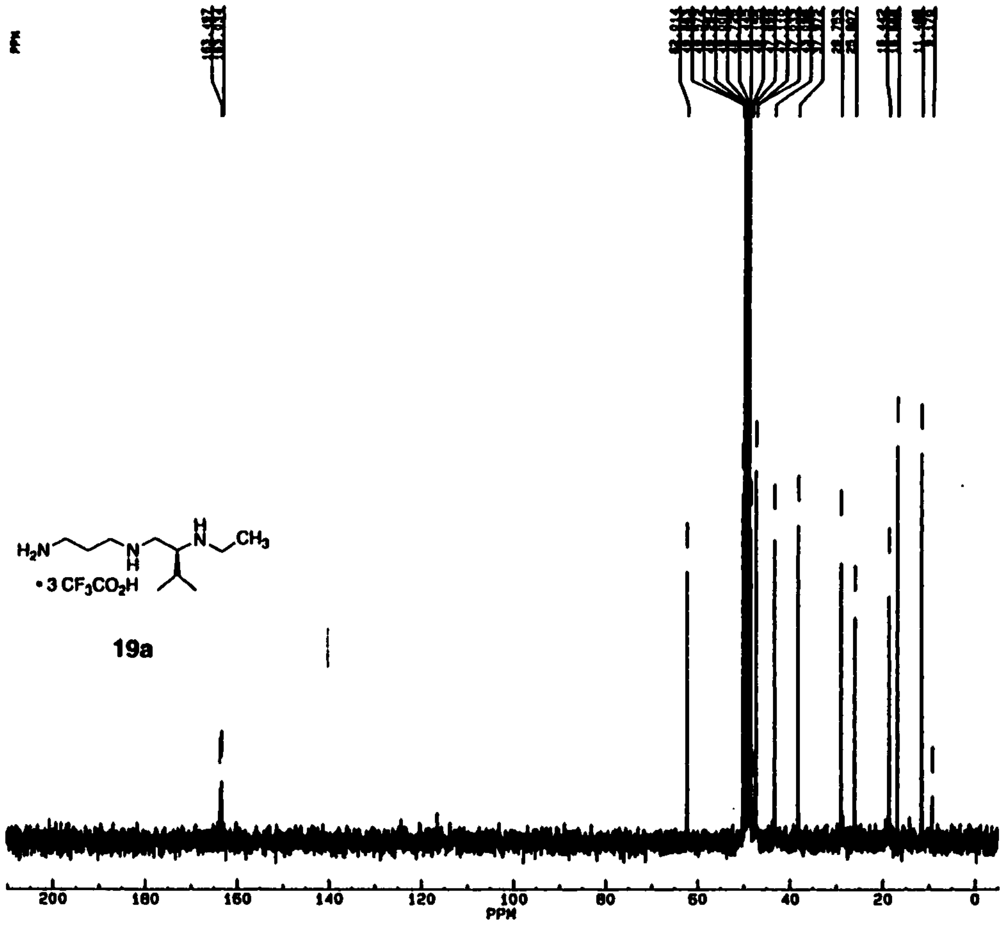
Appendix of NMR Data



H2N
N
H
N
H
CH3
• 3 CF3CO2H
9
8
7
6
5
4
3
2
1
ppm
-0.16
-0.41
12.06
18.56
10.23
16.93
15.27
28.04
0.86

19a



19a

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SY 112.0
O1    6140.000
SI   32768
TD   32768
SW   22727.273
HZ/PT    1.387

PW       2.0
RO        .500
AQ        .721
RG     800
NS    2692
TE     297

FW   28500
O2    5595.000
DP   16H CPD

LB      1.200
GB       .010
CX     20.00
CY      8.00
F1    209.996P
F2     -5.002P
HZ/CM 811.282
PPM/CM 10.750
SR  -1214.53
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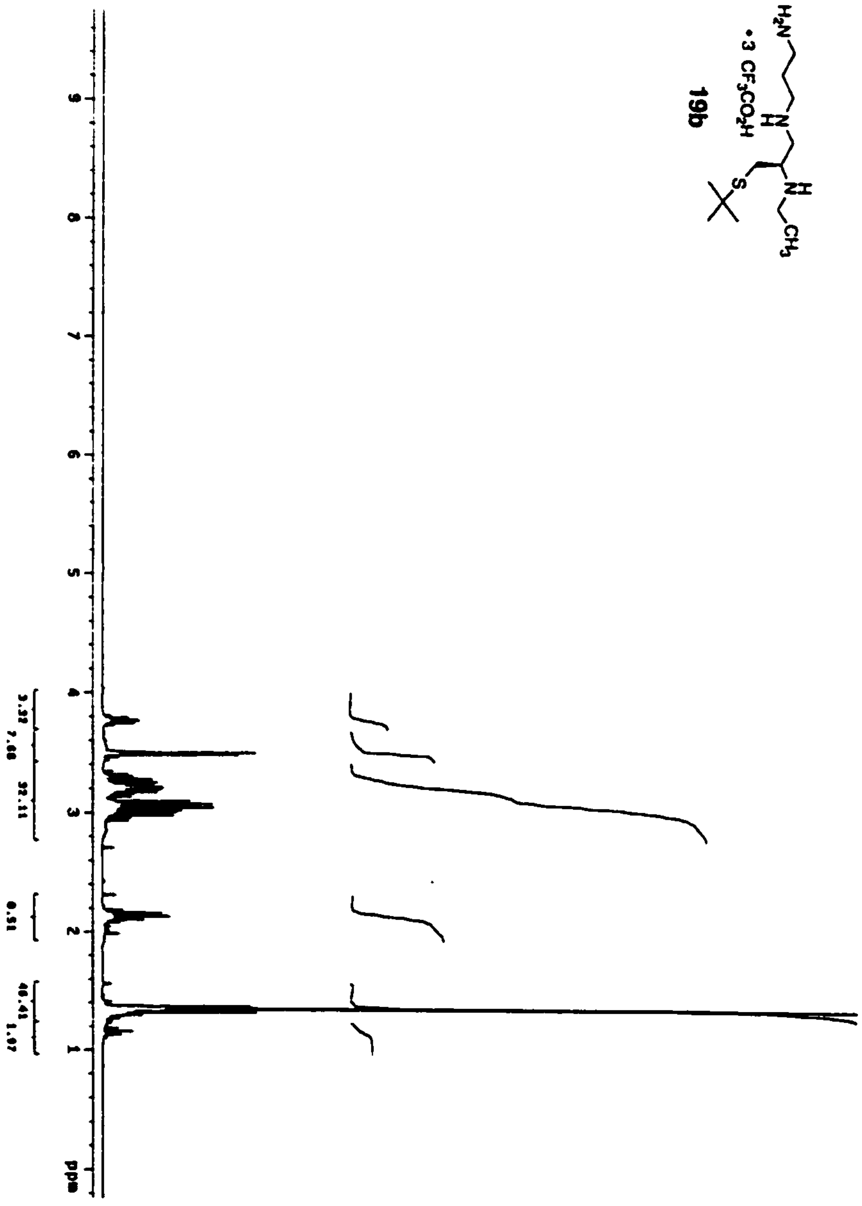
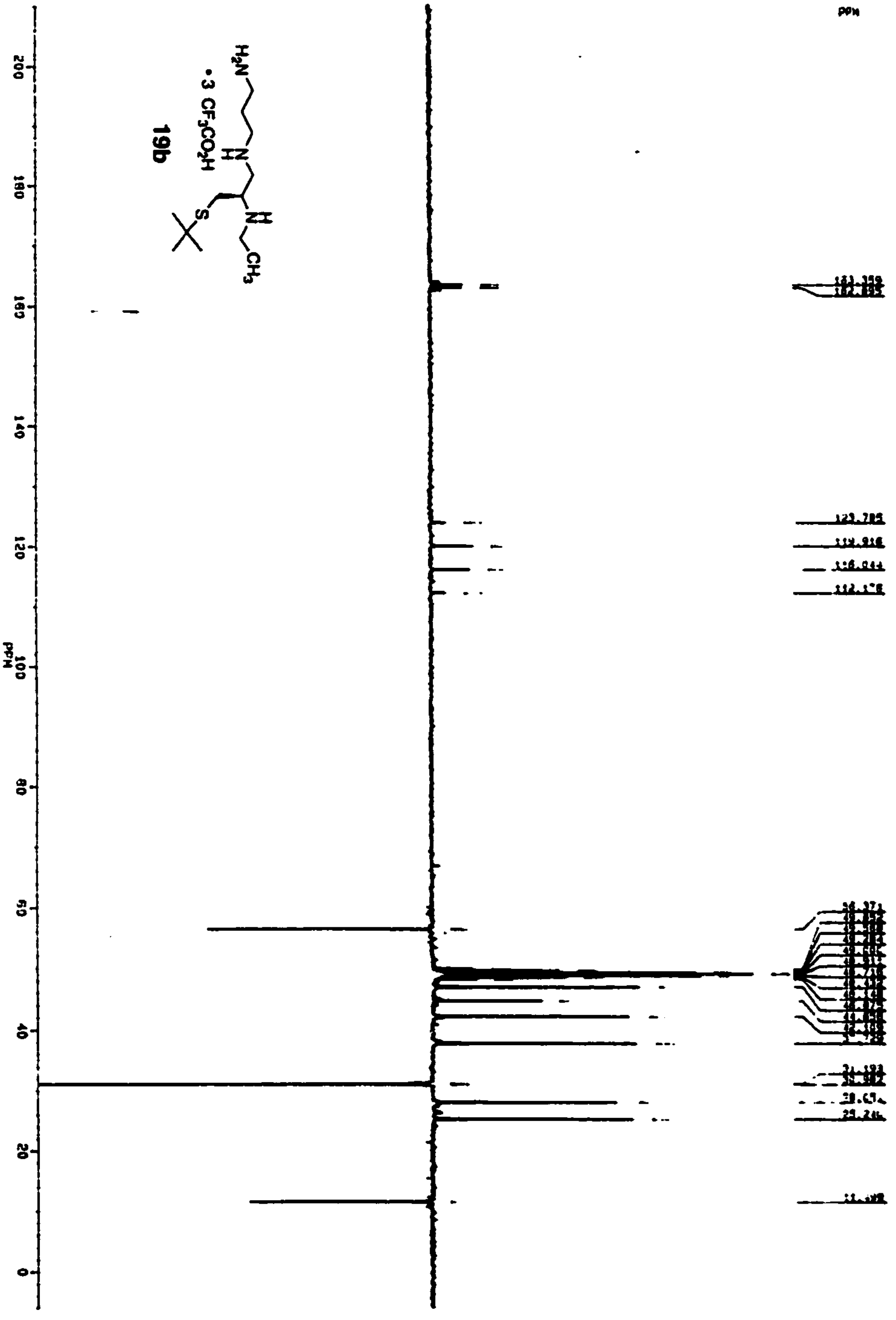
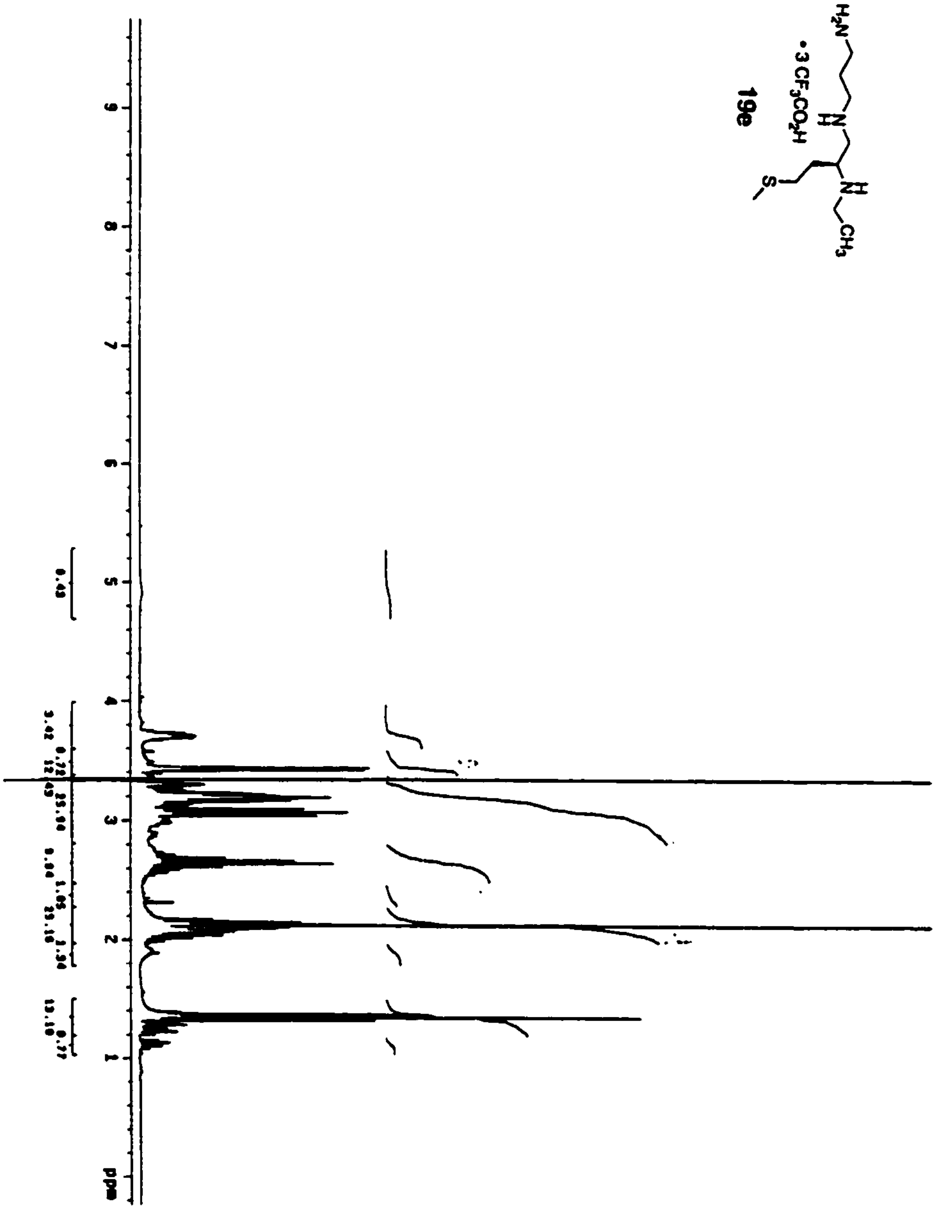

H₂N
H
N
H
N
CH₃
S
•3 CF₃CO₂H
9
8
7
6
5
4
3
2
1
ppm
3.32
7.68
32.11
8.51
40.41
1.97

19b


H2N
H
N
N
H
CH3
S
• 3 CF3CO2H
200
180
160
140
120
100
PPM
80
60
40
20
0
PPM
163.359
123.785
119.916
116.044
112.176

19b



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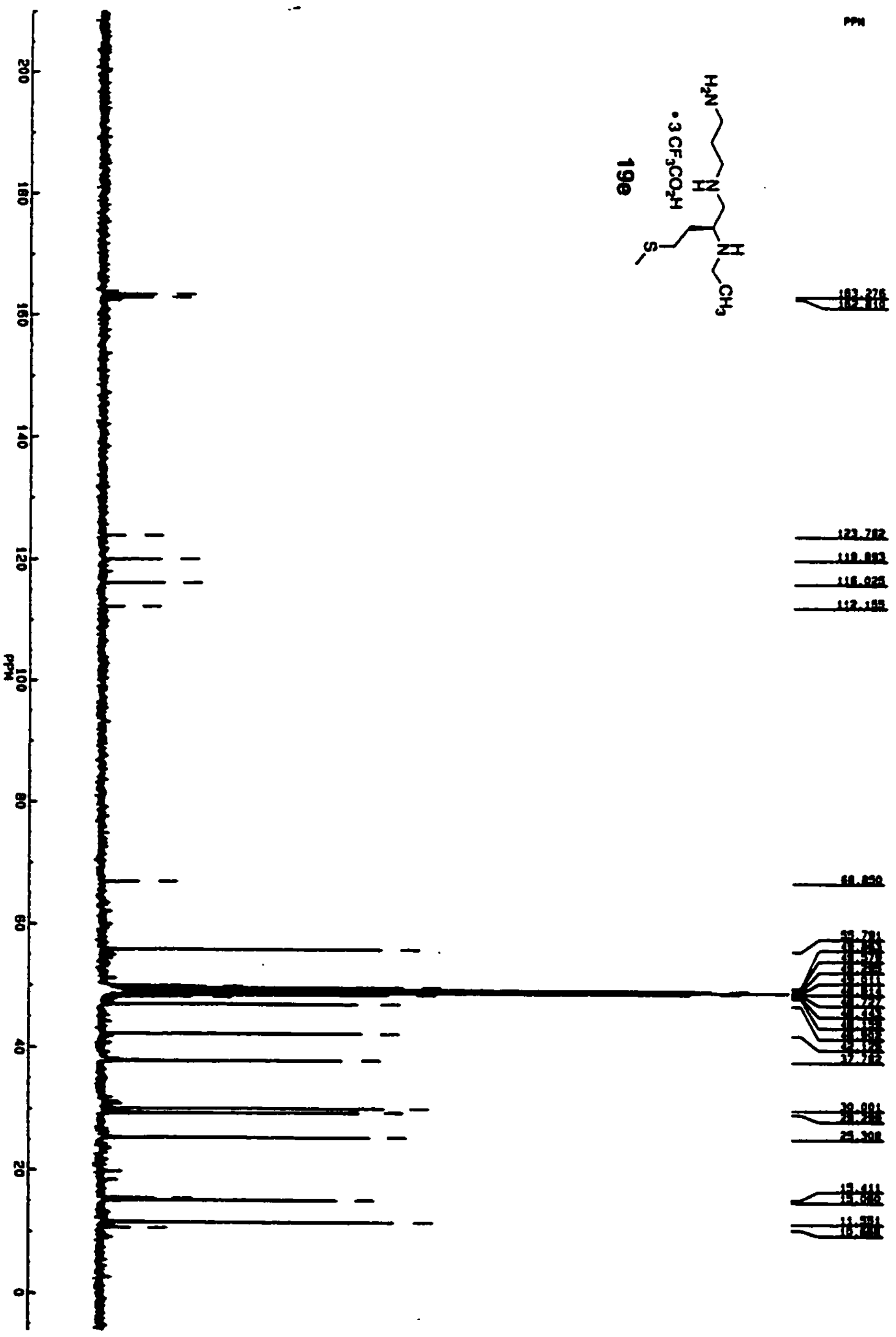
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H
N
H
CH3
S
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PPM
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119.893
116.025
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66.850
55.791
37.782
30.001
25.308
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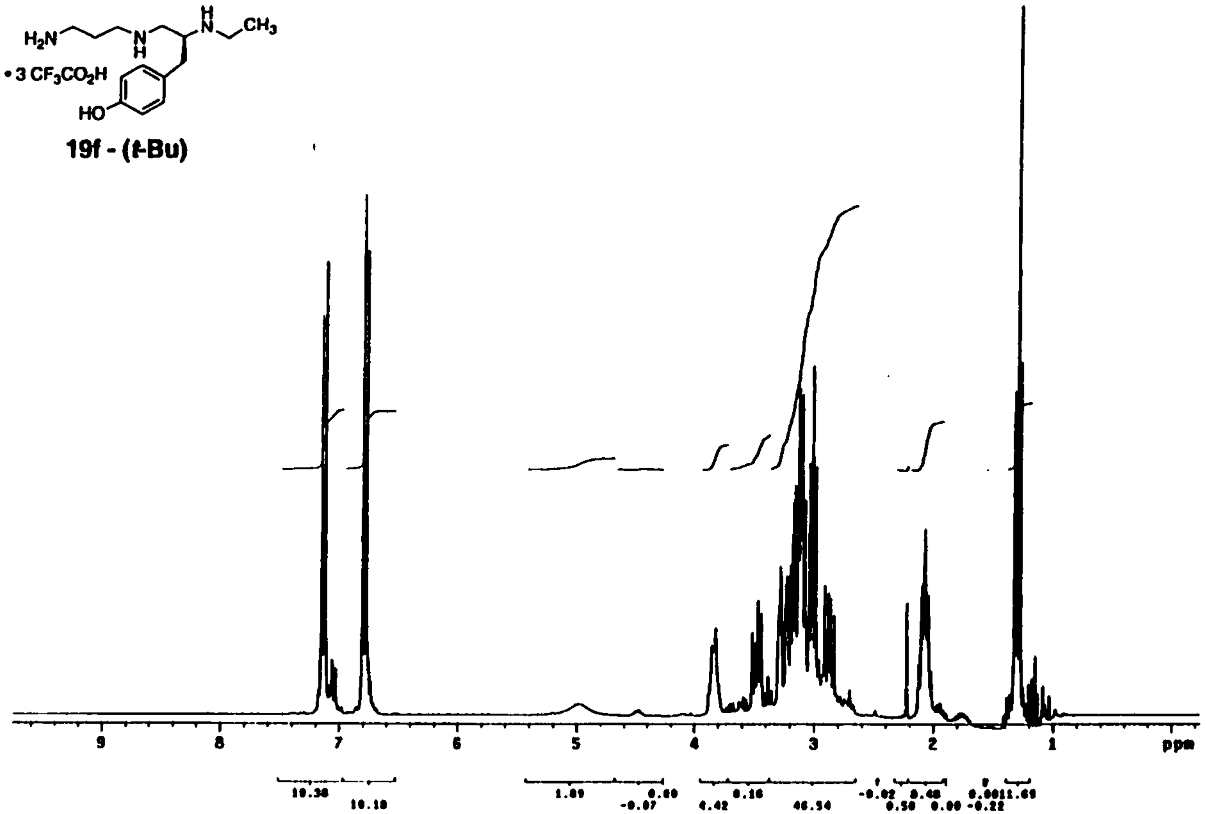
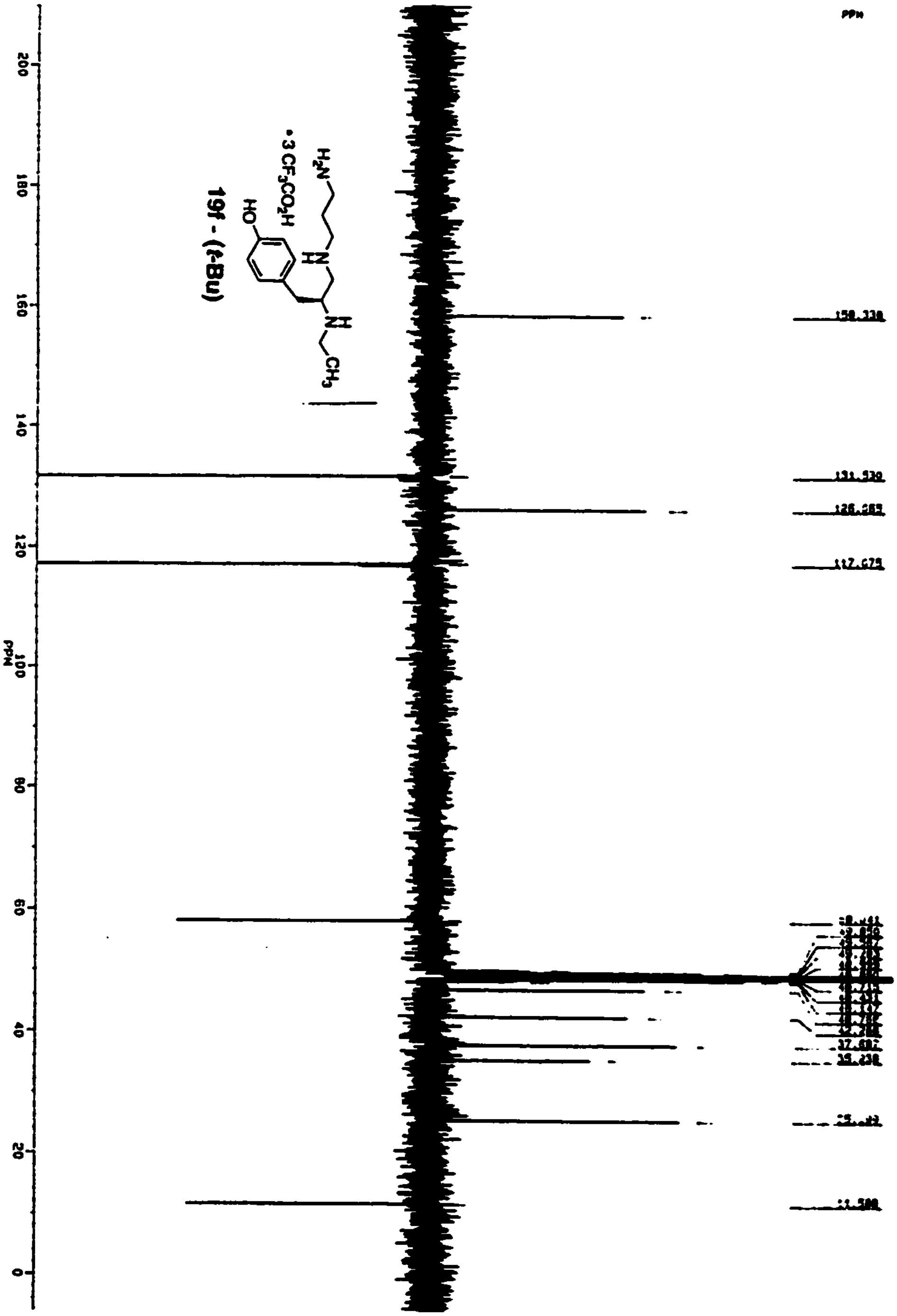
11.551
200
180
160
140
120
100
PPM
80
60
40
20
0

19e


H$_2$N
N H
H N
CH$_3$
• 3 CF$_3$CO$_2$H
HO
9
8
7
6
5
4
3
2
1
ppm
10.30
10.10
1.09
0.00
-0.07
4.42
0.16
46.54
-0.02
0.50
0.40
0.00
0.00
-0.22
11.60

19f - (t-Bu)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N
H
N
H
CH$_3$
HO
•3 CF$_3$CO$_2$H
19f - (t-Bu)
PPM
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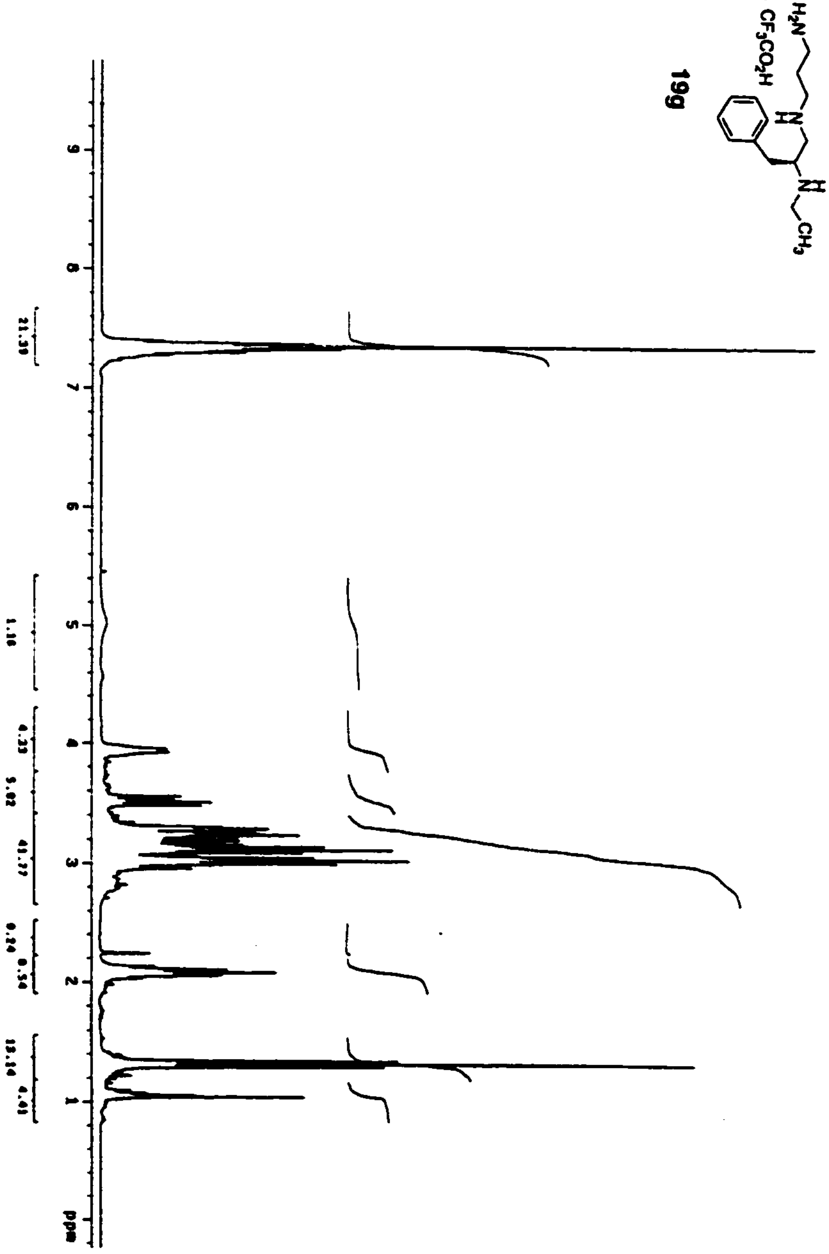
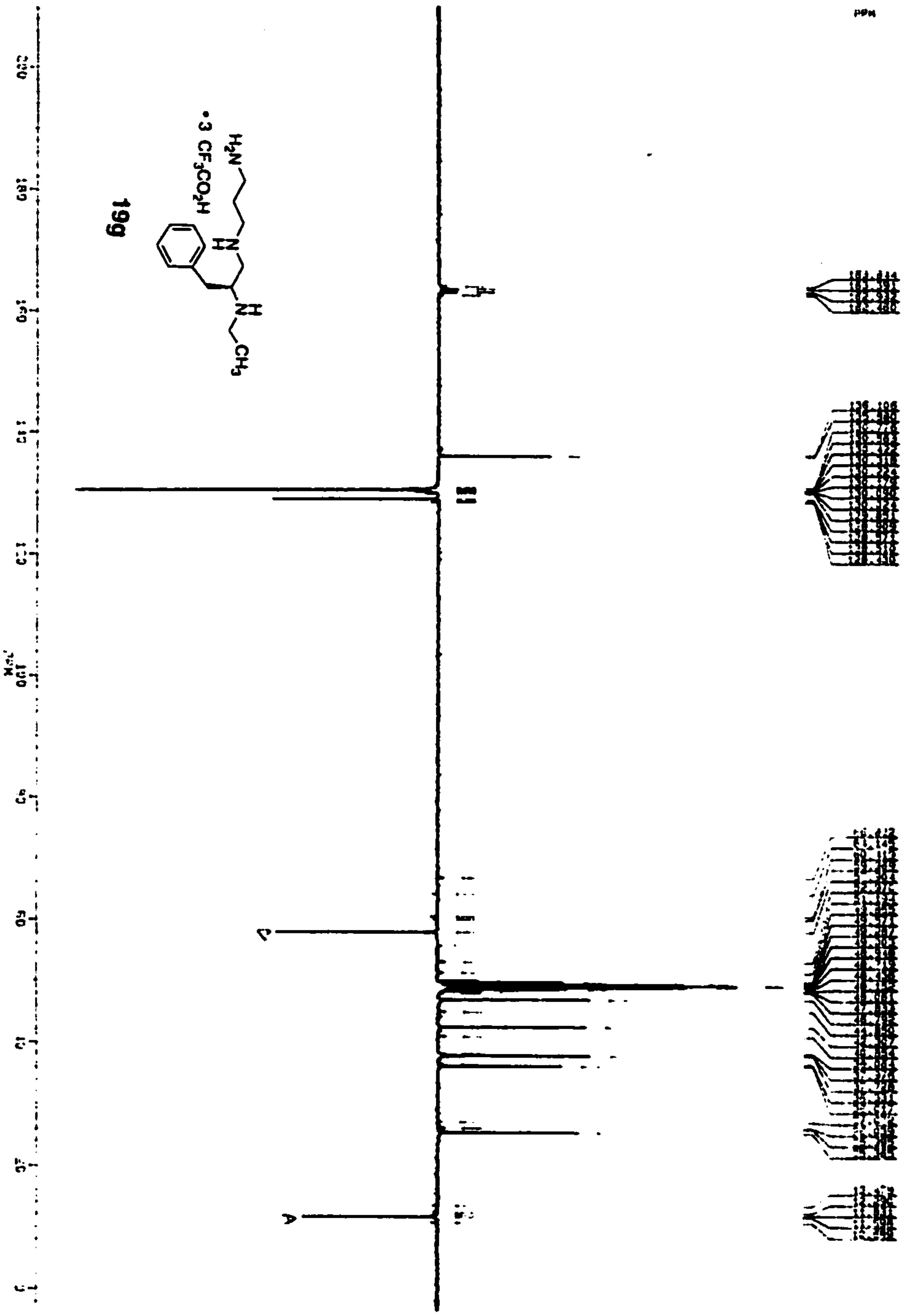
131.530
117.675
37.602
35.238
200
180
160
140
120
100
80
60
40
20
0
PPM


H$_2$N
N
H
N
H
CH$_3$
• 3 CF$_3$CO$_2$H
9
8
7
6
5
4
3
2
1
ppm
21.39
1.16
4.33
5.02
41.77
0.24
8.54
13.14
4.41

19g



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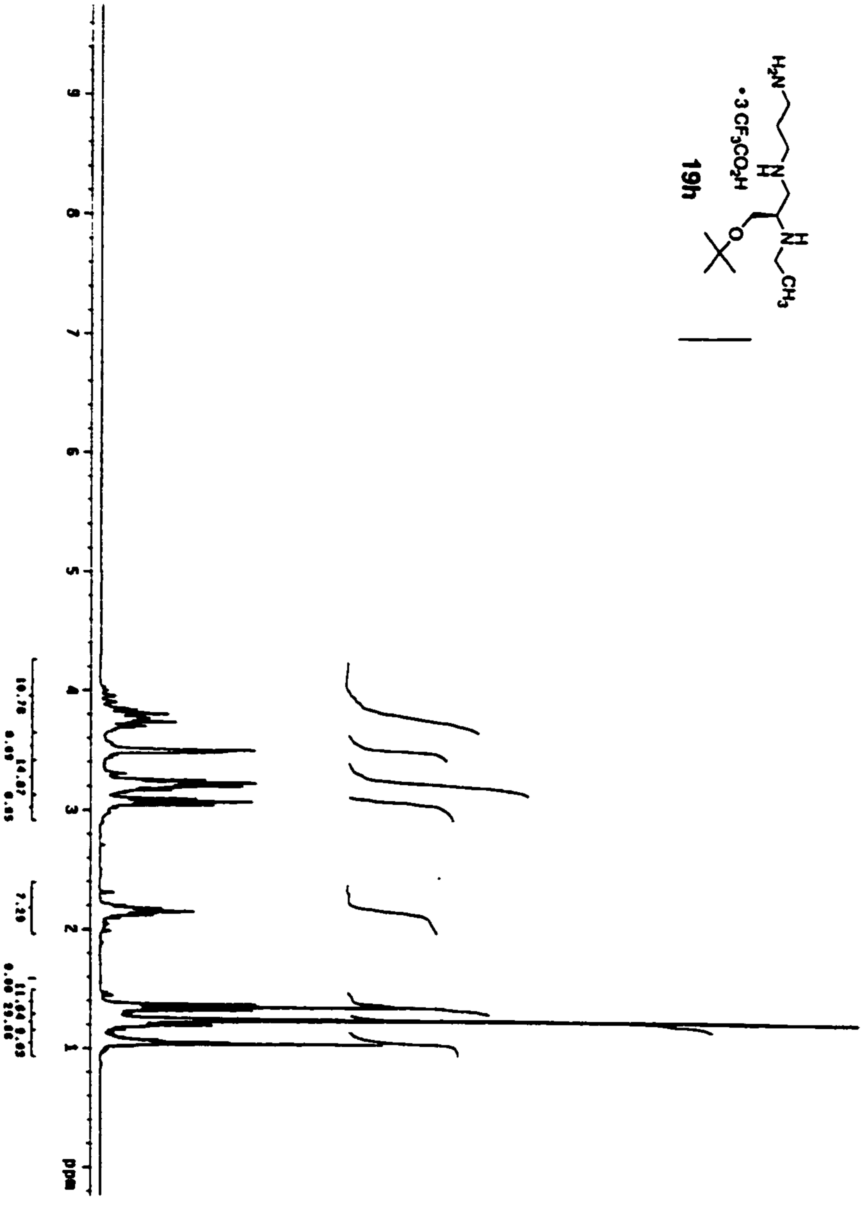
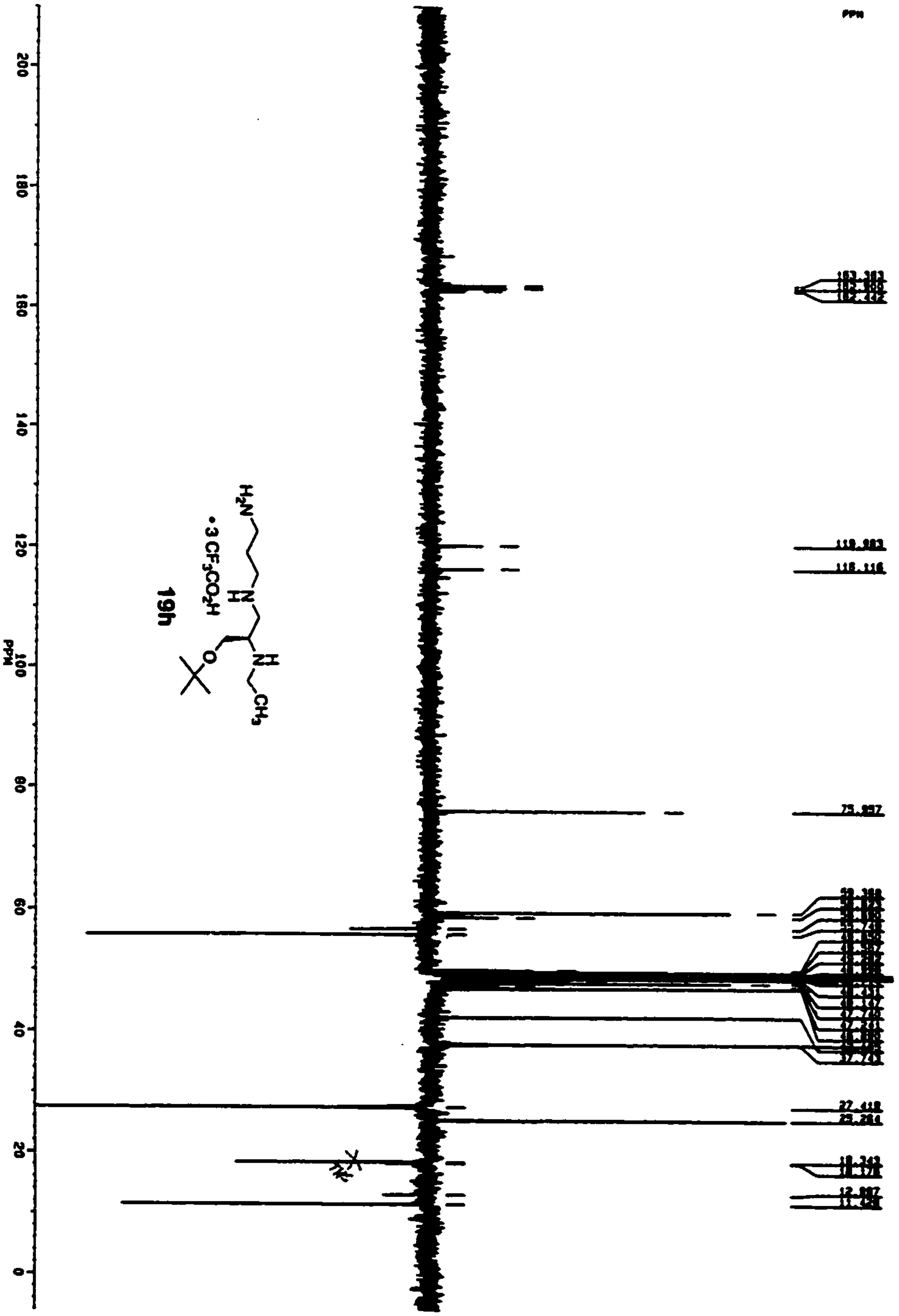
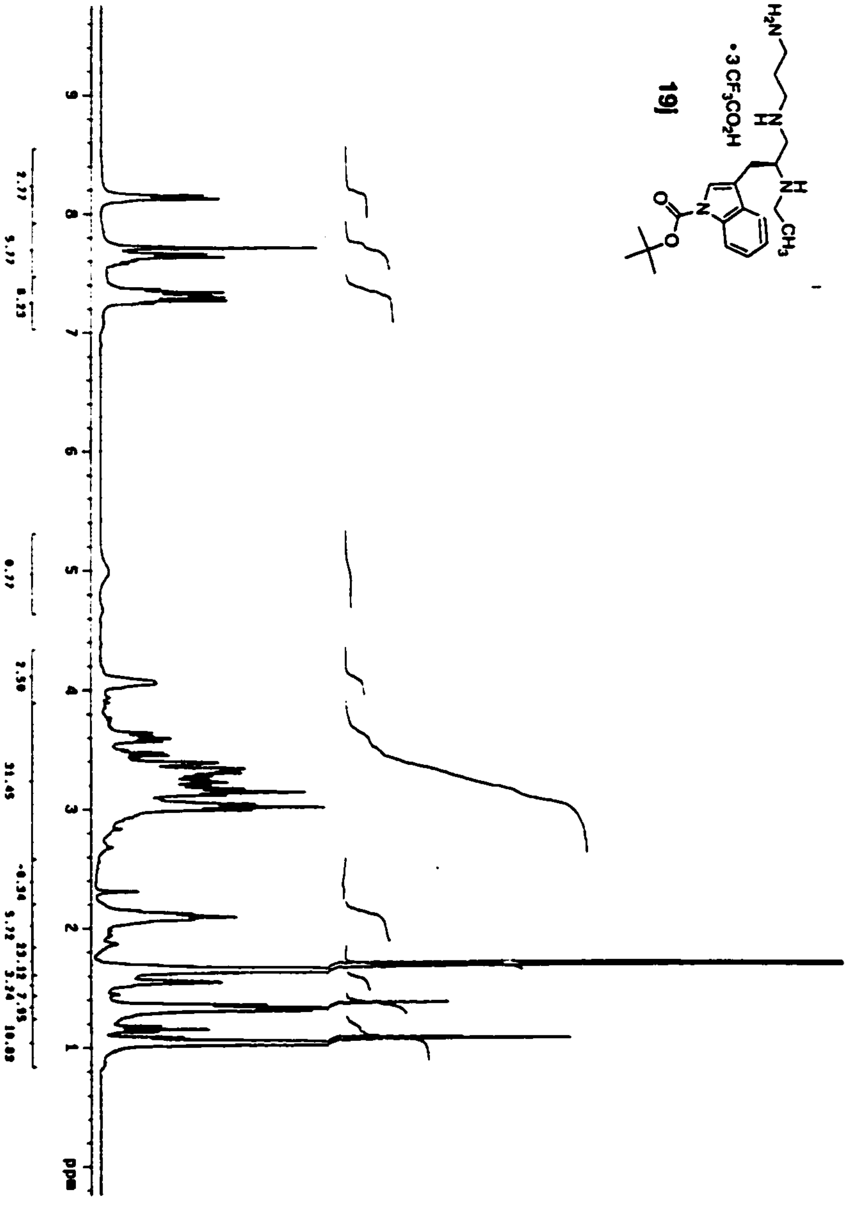


19h



200
180
160
140
120
100
80
60
40
20
0
PPM
PPM
H_2N
N
H
N
H
CH_3
O
•3 CF_3CO_2H
119.983
116.116
75.957
27.418
25.284

19h



19j

H₂N
• 3 CF₃CO₂H
CH₃
9
8
7
6
5
4
3
2
1
ppm
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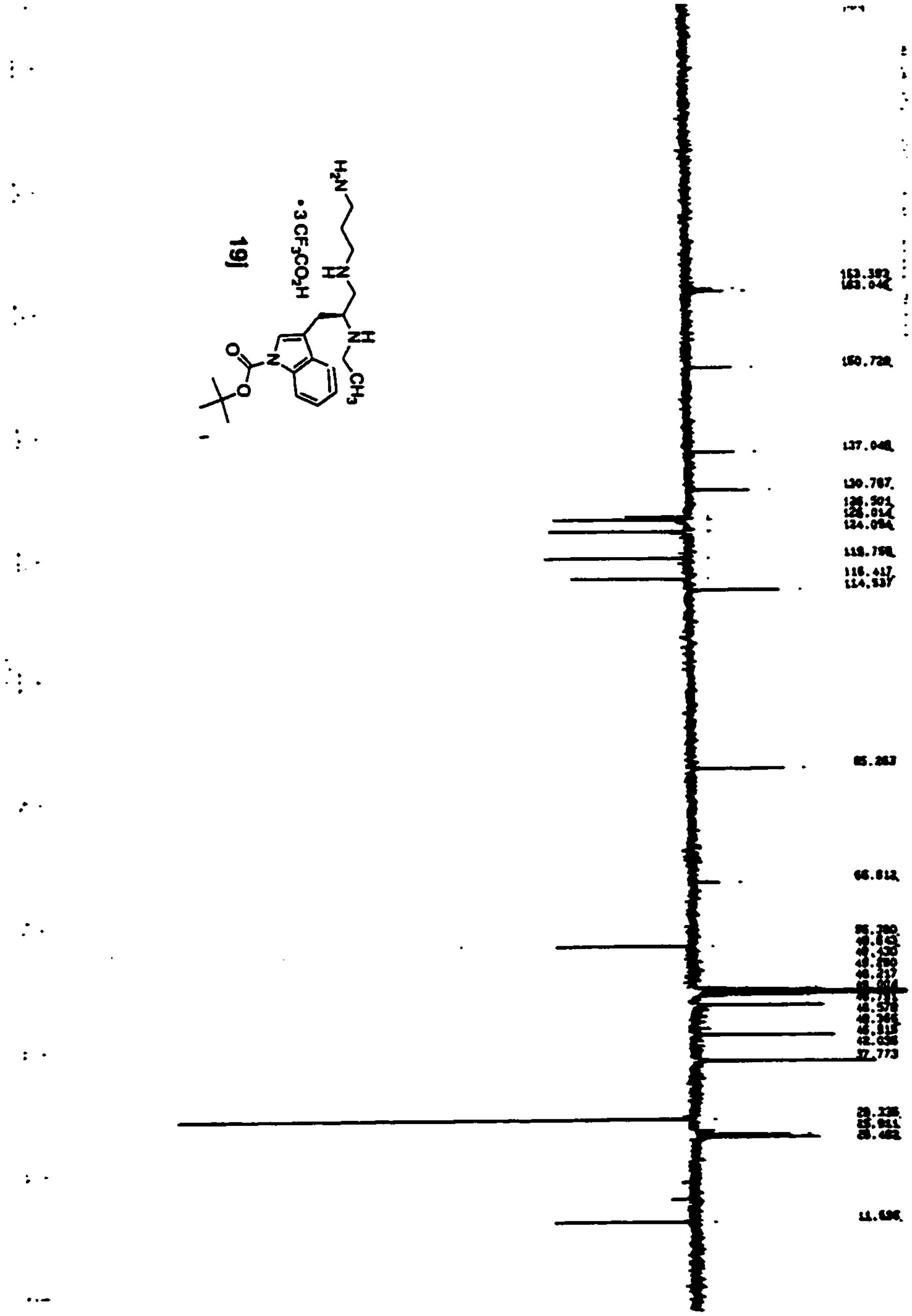
5.77
8.23
0.77
7.50
31.45
-0.34
5.72
23.12
7.95
5.24
10.89



H2N
H
N
H
N
CH3
N
O
O
• 3 CF3CO2H
150.728
137.048
130.787
114.537
37.773

19j