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## ABBREVIATIONS

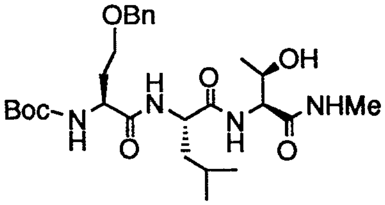
AIBN, azoisobutyronitrile; Boc, t-butoxycarbonyl; Bz, benzyl; DCC, *N,N'*-dicyclohexylcarbodiimide; DCU, *N,N'*-dicyclohexylurea; DMF, dimethylformamide; HF, hydrofluoric acid; Hse, L-homoserine; Leu, leucine; OBn, benzyl ether; THF, tetrahydrofuran; Thr, threonine; TBDMS, *t*-butyldimethylsilyl; TFA, trifluoroacetic acid.

## GENERAL

Proton NMR spectra were acquired on a Bruker 500 MHz spectrometer. Unless otherwise noted, mass spectral analyses were determined by the University of California-Riverside Mass Spectroscopy Facility using standard Fast Atom Bombardment (FAB) protocols. Tritium quantification was obtained in disintegrations per minute (dpm) on a Beckman LS-5000TD scintillation counter (further details are provided in the experimental secttion of the paper). HPLC purification and analysis were on a Beckman Dual Channel System operating with Sytem Gold software with an analytical grade, C-18 reverse phase column (25cm x 4.6mm).

## SYNTHETIC PROTOCOLS AND PHYSICAL DATA

*Synthesis of Boc-Hse(OBn)-Leu-Thr-NHMe* :



Boc-Hse(OBn) (50.3 mg, 163 µmoles) was dissolved in 600 µL THF. DCC (36.7 mg, 178 µmoles, 1.1 eq) and *p*-nitrophenol (25.8 mg, 186 µmoles, 1.14 eq) were added. The solution was stirred for four hours and then filtered to remove DCU. The activated ester was briefly purified via silica gel chromatography and used immediately (53.6 mg, 125 µmoles). TFA-Leu-Thr-NHMe (51.4 mg, 149 µmoles, 1.2 eq) was dissolved in 600 µL DMF and combined with triethylamine (39 µL, 282 µmoles, 2.2 eq). This solution was added to the activated Boc-Hse(OBn) *p*-nitrophenol ester and stirred for 16 hours. The resultant tripeptide was purified by

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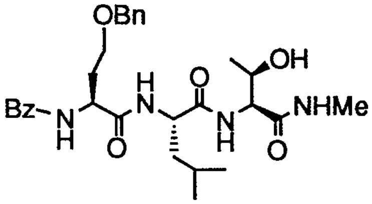
silica gel chromatography to yield 65 mg (121 µmoles, 68 % yield) Boc-Hse(OBn)-Leu-Thr-NHMe. TLC: $R_f$ = 0.45 in 5/1 chloroform/methanol. MS calculated for $C_{27}H_{44}N_4O_7$ ($MH^+$) 537.6, observed 537.3. $^1H$ NMR, $CDCl_3$, (δ ppm): 7.31 (m, 5H), 7.23 (d, 1H, J = 8.2 Hz), 7.09 (d, 1H, J = 6.7 Hz), 6.91 (d, 1H, J = 5.3 Hz), 4.48 (s, 2H), 6.06 (d, 1H, J = 3.4 Hz), 4.36 (d, 2H, J = 7.1 Hz), 4.23 (dd, 1H, J = 11.6 Hz, 5.2 Hz), 3.61 (m, 1H), 3.66 (m, 1H), 2.08 (m, 1H), 2.77 (d, 3H, J = 4.8 Hz), 1.99 (m, 1H), 1.63 (m, 2H), 1.25 (s, 1H), 1.43 (s, 9H), 1.49 (m, 1H), 1.12 (d, 3H, J = 6.4 Hz), 0.89 (dd, 6H, J = 9.3 Hz, 6.3 Hz).

*Synthesis of Bz-Hse(OBn)-Leu-Thr-NHMe:*



Boc-Hse(OBn)-Leu-Thr-NHMe (61.8 mg, 115 µmoles) was dissolved in 2 mL dichloromethane and 2 mL trifluoroacetic acid and stirred for 20 minutes. The TFA was removed under a stream of nitrogen and the resultant oil was lyophilized from water to afford a fluffy white powder which was redissolved in 550 µL DMF. The *p*-nitrophenyl ester of benzoic acid was added (33 mg, 136 µmoles, 1.2 eq) along with triethylamine (23 µL, 166 µmoles, 1.5 eq). The reaction was complete after 6 hours at which time the DMF was removed under vacuum. The product was purified by silica gel chromatography to yield 48 mg (84.9 µmoles, 76% yield) Bz-Hse(OBn)-Leu-Thr-NHMe. TLC: $R_f$ = 0.5 in 5/1 chloroform/methanol. MS calculated for $C_{29}H_{40}N_4O_6$ ($MH^+$) 541.6, observed 541.3. $^1H$ NMR, $CDCl_3$, (δ ppm): 8.10 - 6.95 (m, 14H), 4.71 (dd, 1 H, J = 5.1 Hz, 12.0 Hz), 4.51 (s, 2H), 4.52-4.45 (m, 1H), 4.39 (m, 2H), 4.10 (m, 1H), 3.84 (m, 1H), 3.74 (m, 1H), 2.78 (d, 3H, J = 4.8 Hz), 2.22 (m, 2H), 1.69 (m, 1H), 1.60 (m, 1H), 1.51 (m, 1H), 1.14 (d, 3H, J = 6.4 Hz), 0.88 (d, 3H, J = 6.5 Hz), 0.86 (d, 3H, 6.4 Hz).

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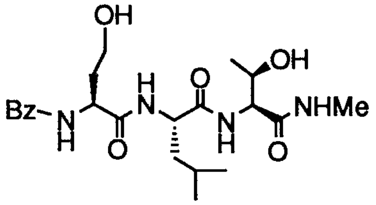
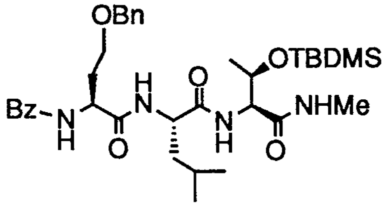
*Synthesis of Bz-Hse-Leu-Thr-NHMe:*



Bz-Hse(OBn)-Leu-Thr-NHMe (14 mg, 26 μmoles, 1 eq) was dissolved in 1 mL methanol. The flask was purged with nitrogen. Approximately 2 mg palladium over carbon were introduced to the flask and the flask was purged again and evacuated. Hydrogen gas was introduced into the flask from a balloon and the reaction was stirred for thirty minutes. The solution was filtered over celite and dried down to afford 10 mg (22.2 μmoles, 86 % yield) Bz-Hse-Leu-Thr-NHMe. TLC: $R_f$ = 0.46 in 5/1 chloroform/methanol. MS calculated for $C_{22}H_{32}N_4O_6$ ($MH^+$) 450.5 observed 451.3. $^1$H NMR, $CDCl_3$, (δ ppm): 8.10 (d, 1H, J = 6.7 Hz), 7.75 (m, 3H), 7.53 (d, 1H, J = 8.2 Hz), 7.49-7.37 (m, 4H), 7.24 (m, 1H), 4.71 (m, 1H), 4.34 (m, 1H), 4.25 (m, 2H), 3.68 (m, 2H), 2.72 (d, 3H, J = 4.7 Hz), 2.1 (m, 1H), 1.9 (m, 1H), 1.67 - 1.53 (m, 3H), 1.1 (m, 3H), 0.88 (d, 3H, J = 4.4 Hz), 0.83 (d, 3H, J = 4.2 Hz).

*Synthesis of Bz-Hse(OBn)-Leu-Thr(TBDMS)-NHMe:*



Bz-Hse(OBn)-Leu-Thr-NHMe (48 mg, 88.8 μmoles, 1 eq) was dissolved in 1 mL anhydrous acetonitrile. The solution was chilled in an ice bath and pyridine (34 μL, 422 μmoles, 5 eq) and TBDMS-triflate (48 μL, 209 μmoles, 2.5 eq) were added. The reaction was slowly warmed to room temperature and stirred for 90 minutes. The reaction was then quenched with saturated ammonium chloride and the product was extracted into chloroform. The product was purified by silica gel chromatography to yield Bz-Hse(OBn)-Leu-Thr(TBDMS)-NHMe (40 mg, 61.1 μmoles, 69% yield). TLC: $R_f$ = 0.32 in 10/1 chloroform/methanol. MS calculated for $C_{35}H_{54}N_4O_6Si$ ($MH^+$) 655.9, observed 655.4. $^1$H NMR, $CDCl_3$, (δ ppm): 7.87 - 6.77 (m, 14 H), 4.66 (m, 1H), 4.45 (s, 2H), 4.43 (m, 1H), 4.15 (m, 2H), 3.95 (m, 1H), 3.71 (m, 1H), 2.71 (d, 3H,

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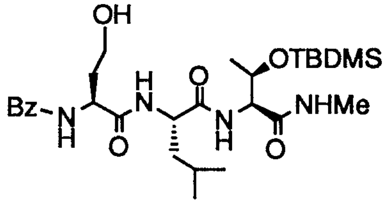
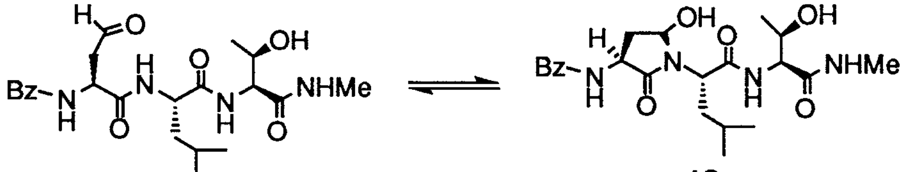
J = 4.7 Hz), 2.17 (m, 1H), 2.11 (m, 1H), 1.60 - 1.41 (m, 3H), 1.01 (d, 3H, J = 6.3 Hz), 0.81 - 0.74 (m, 15 Hz), 0.02 (s, 3H), -0.02 (s, 3H).

*Synthesis of Bz-Hse-Leu-Thr(TBDMS)-NHMe:*



Bz-Hse(OBn)-Leu-Thr(TBDMS)-NHMe (13 mg, 19.9 μmoles) was dissolved in approximately 1 mL methanol. Reaction vessel was purged with nitrogen and $Pd(OH)_2$ was added. Reaction vessel was purged again and evacuated. Hydrogen gas was introduced from a balloon for one hour with stirring. The reaction was monitored by TLC and, when complete, filtered over celite and dried down to afford Bz-Hse-Leu-Thr(TBDMS)-NHMe (10 mg, 17.7 μmoles, 89.0 % yield). TLC: $R_f$ = 0.56 in 9/1 ethyl acetate/methanol. MS calculated for $C_{28}H_{48}N_4O_6Si$ ($MH^+$) 565.8, observed 565.3. $^1H$ NMR, $CDCl_3$, (δ ppm): 7.83 - 7.44 (m, 6H), 6.85 (d, 1H, J = 4.8 Hz), 4.95 (m, 1H), 4.41 (m, 1H), 4.33 (m, 1H), 3.85(m, 1H), 3.77 (m, 1H), 2.80 (d, 3H, J = 4.8 Hz), 2.25 (m, 1H), 1.94 (m, 1H), 1.70 (m, 3H), 1.06 (d, 3H, J = 6.3 Hz), 0.89 (m, 15 H), 0.13 (s, 3H), 0.11 (s, 3H).

*Synthesis of **9** and **10**:*



**9** **10**

Chromium (VI) trioxide (23.3 mg, 233 μmoles, 13.1 eq) and pyridine (35 μL, 434 μmoles, 24.5 eq) were combined with 0.5 mL anhydrous dichloromethane and chilled to 0°C. The yellow-brown mixture was stirred for fifteen minutes and a 0.5 mL solution of Bz-Hse-Leu-Thr(TBDMS)-NHMe (10.0 mg, 17.7 μmoles, 1 eq) in anhydrous dichloromethane was added via cannula. The solution turned black and was allowed to stir for 20 minutes at 0°C. The solution was immediately passed over several columns of silica gel in 9/1 ethyl acetate/methanol to completely remove the chromium. The remaining residue (3.1 mg) was dissolved in 30 μL

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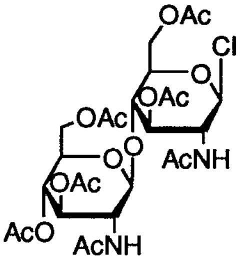
acetonitrile and a 2 % HF solution in acetonitrile (11.6 µL) was added. After 60 minutes, an additional 5.5 µL HF solution was added. The reaction was stirred for 90 minutes and then brought to dryness. The product was purified by analytical HPLC on a 10 % to 30 % gradient of acetonitrile in water (0.1 % TFA). MS calculated for $C_{27}H_{44}N_4O_7$ ($MH^+$) 449.5, observed 449.

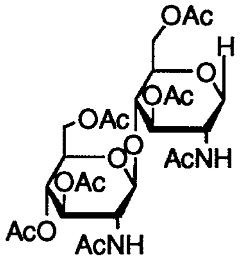
Additionally, the impure product (1 mg, 2.2 µmoles, 1eq) was treated with excess sodium borohydride (0.88 mg, 23 mmoles) in methanol for forty five minutes and reexamined by HPLC. The product was quantitatively reduced to Bz-Hse-Leu-Thr-NHMe.

*Synthesis of 2-acetamido-3,6-Di-O-acetyl-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxygluco-pyranosyl)-1-chloro-1-deoxy-D-glucopyranoside:*



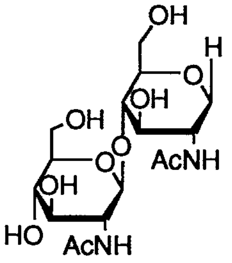
The disaccharide 2-acetamido-3,6-Di-O-acetyl-1-chloro-1-deoxy-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxyglucopyranosyl)-D-glucopyranoside was prepared from octaacetyl-chitobiose (56.5 mg, 83.7 µmoles) as previously described (Hiraizumi et al., 1993). Further purification was performed on silica gel for a final yield of 26 mg (39.8 µmoles, 47.5 % yield). TLC: $R_f$ = 0.49 in 9/1 ethyl acetate/methanol. $^1H$ NMR, $CDCl_3$, (δ ppm): 6.15 - 6.10 (m, 2 H, H-1 and N-H'), 5.94 (d, 1H, J = 7.5 Hz, N-H), 5.29 (m, 1H, H-3), 5.21(m, 1H, H-3'), 5.03 (m, 1H, H-4'), 4.61 (m, 1H, H-1'), 4.45 - 4.35 (m, 4H, H-2, H-6A, H-6B, H-6A'), 4.18 (m, 1H, H-5), 4.02 (m, 1H, H-6B'), 3.87 - 3.77 (m, 2H, H-2' and H-4), 3.65 (m, 1H, H-5'), 2.15 - 1.94 (m, 21H, acetyl $CH_3$'s).

*Synthesis of 2-acetamido-3,6-Di-O-acetyl-1,5-anhydro-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxyglucopyranosyl)-2-deoxy-D-glucitol:*



The heptaacetyl chloride (5.4 mg, 8.3 µmoles) was dissolved in dry toluene (1.1 mL) with 2 mg AIBN. Tributyl tin hydride (64 µL) was added and the reaction was heated at 90 °C for 11 hours. Toluene was removed and the product was separated from remaining starting material by silica gel chromatography for a final yield of 1.5 mg (2.4 µmoles, 29.2 % yield). TLC: $R_f$ = 0.30 in 9/1 ethyl acetate/methanol. $^1$H NMR, $CDCl_3$, ($\delta$ ppm): 5.99 (d, 1H, J = 9.0 Hz, N-H'), 5.87 (d, 1H, J = 7.4 Hz, N-H), 5.16 (m, 1H, H-3'), 5.06 (m, 1H, H-4'), 4.93 (m, 1H, H-3), 4.51 (d, 1H, J = 8.4 Hz, H-1'), 4.39 - 4.27 (m, 3H, H-6A, H-6B, H-6A'), 4.14 - 4.10 (m, 2H, H-6B', H-2), 4.03 (d, 1H, J = 12.4 Hz, H-1A), 3.93 (m, 1H, H-2'), 3.64 (m, 2H, H-4, H-5'), 3.48 (m, 1H, H-5), 3.12 (m, 1H, H-1B), 2.19 - 1.94 (m, 21H, acetyl $CH_3$'s).

*Synthesis of 2-acetamido-1,5-anhydro-4-O-(2-acetamido-2-deoxyglucopyranosyl)-2-deoxy-glucitol:*



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The protected disaccharide 2-acetamido-3,6-Di-O-acetyl-1,5-anhydro-4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxyglucopyranosyl)-2-deoxy-D-glucitol (1.5 mg, 2.4 µmoles) was dissolved in 0.5 mL 3/2 chloroform/methanol. A 50 µL aliquot of an anhydrous solution of sodium methoxide (750 mM) was added to the disaccharide solution. After 20 minutes, the reaction was complete by TLC. The solution was neutralized with Dowex-50 pyridinium-HCl, filtered and brought to dryness to quantitatively yield the deprotected disaccharide. TLC: $R_f$ =

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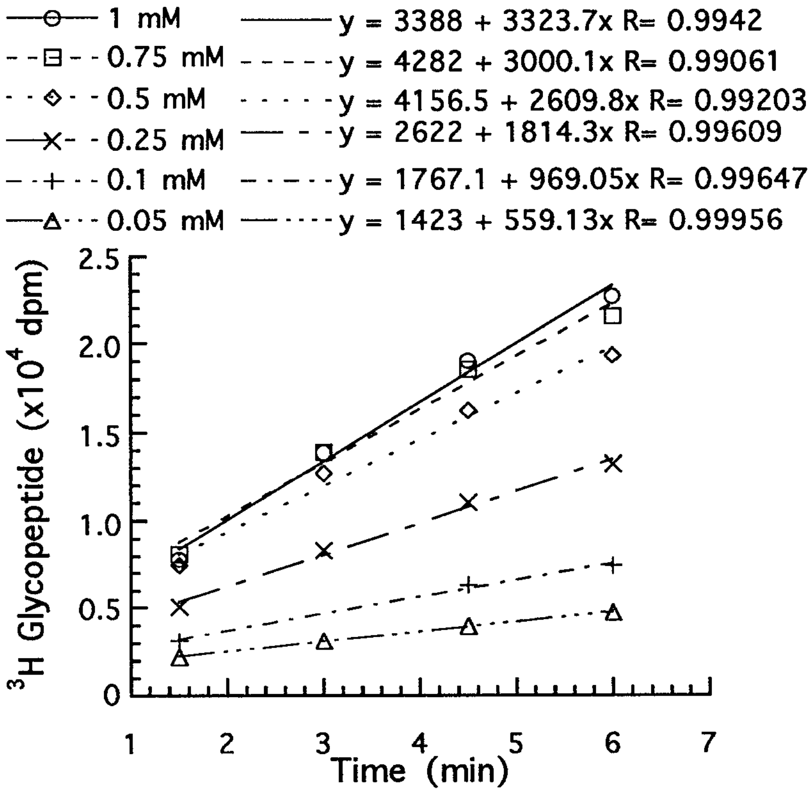
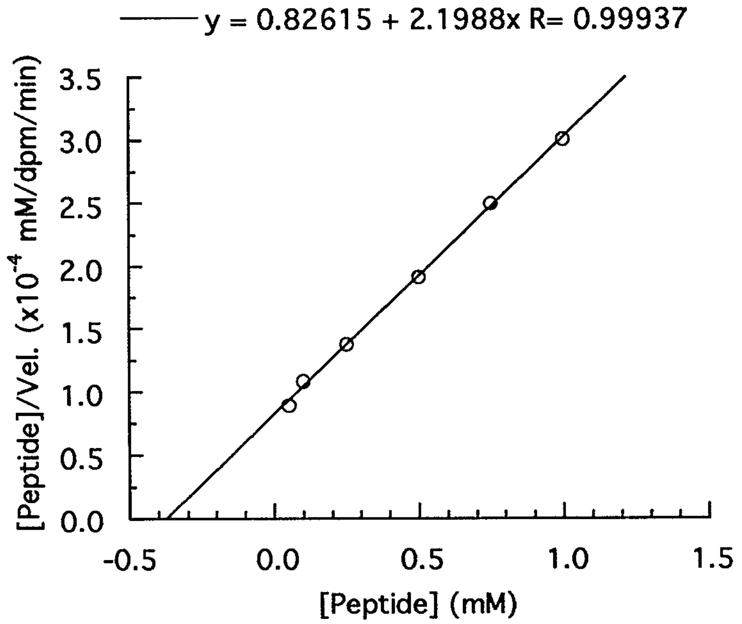
0.08 in 65/35/4/4 chloroform/methanol/water/15 M ammonium hydroxide. MS calculated for $C_{16}H_{28}N_2O_{12}$ ($MH^+$) 409, ($MNa^+$) 431, observed 409 and 431. $^1H$ NMR, $d_6$-DMSO, ($\delta$ ppm): 7.74 (d, 1H, J = 7.83 Hz, N-H), 7.68 (d, 1H, J = 9.52 Hz, N-H'), 4.29 (d, 1H, J = 8.51 Hz, H-1'), 3.74 - 3.17 (br. m, 11H), 3.03 (m, 2H), 2.91 (m, 1H, H-1B), 1.74 (s, 3H, acetyl $CH_3$), 1.71 (s, 3H, acetyl $CH_3$). $^1H$ NMR Chemical Shifts obtained from Double Quantum Filtered COSY, $d_6$-DMSO, ($\delta$ ppm): 7.74 (N-H), 7.68 (N-H'), 4.29 (H-1'), 3.64 (H-1A), 3.55 (H-2), 3.41 (H-2'), 3.38 (H-3), 2.84 (H-1B).

## KINETIC DATA FOR **1** AND **2**

Data for the determination of $K_M$ and $V_{max}$ were obtained as described in the experimental section. The following graphs represent the data used to obtain these kinetic constants which are listed in Table 1 of the preceding paper.

# Kinetic Data for Bz-Asn-Leu-Thr-NHME with Manganese





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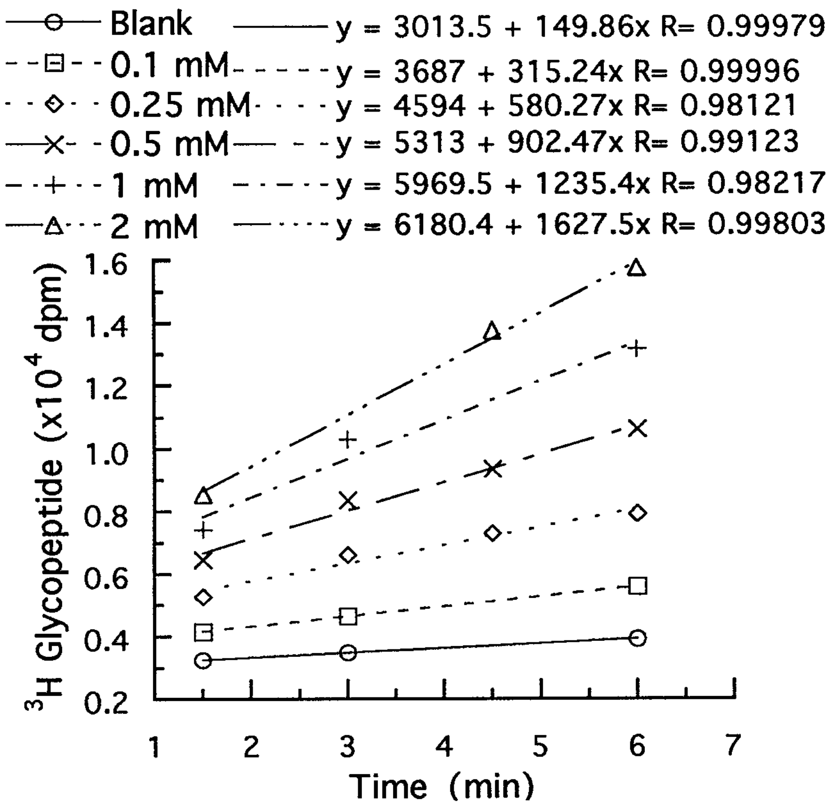
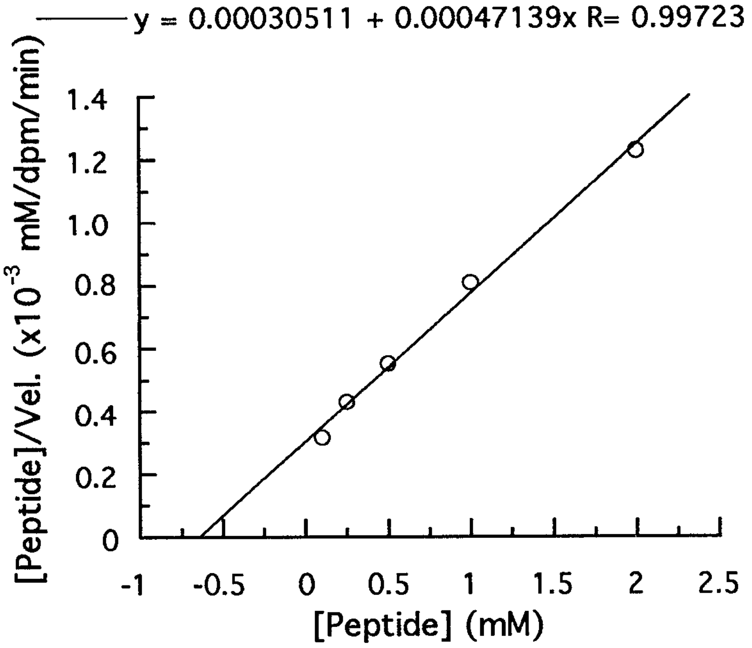
## Kinetic Data for Bz-Asn-Leu-Thr-NHMe with Iron





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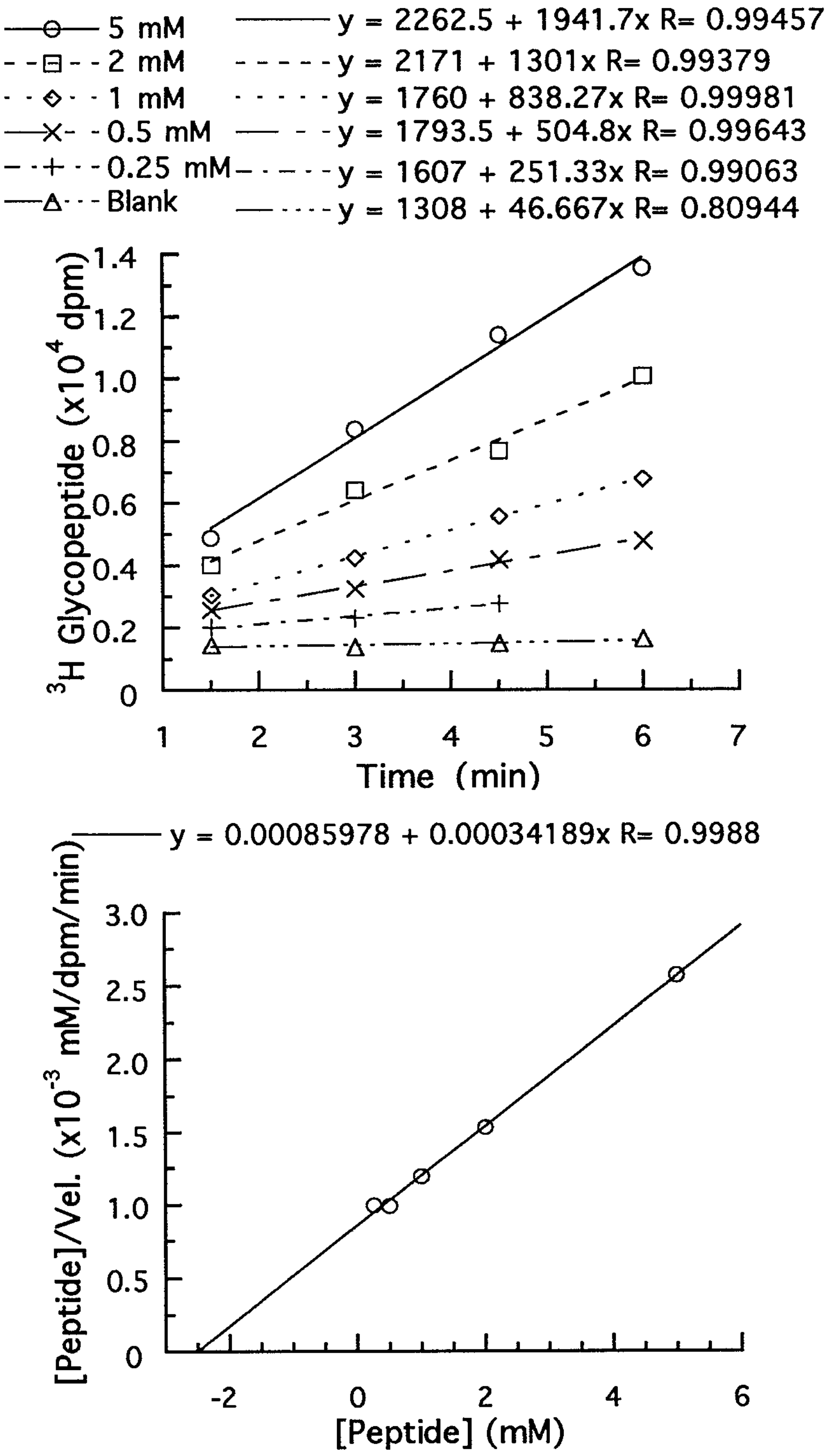
## Kinetic Data for Bz-Asn-Leu-Thr-NHMe with Magnesium



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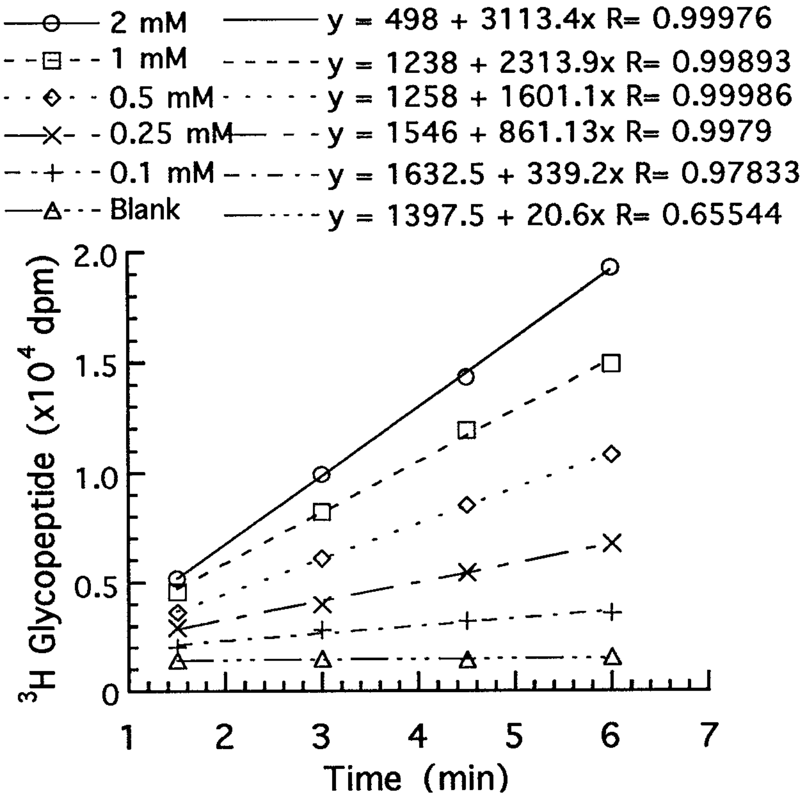
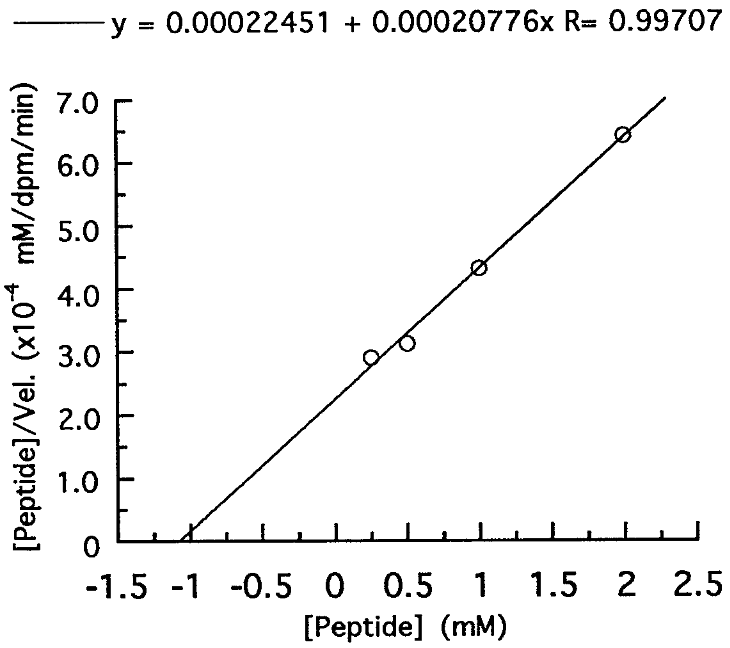
## Kinetic Data for Bz-Asn-Leu-Thr-NHMe with Calcium





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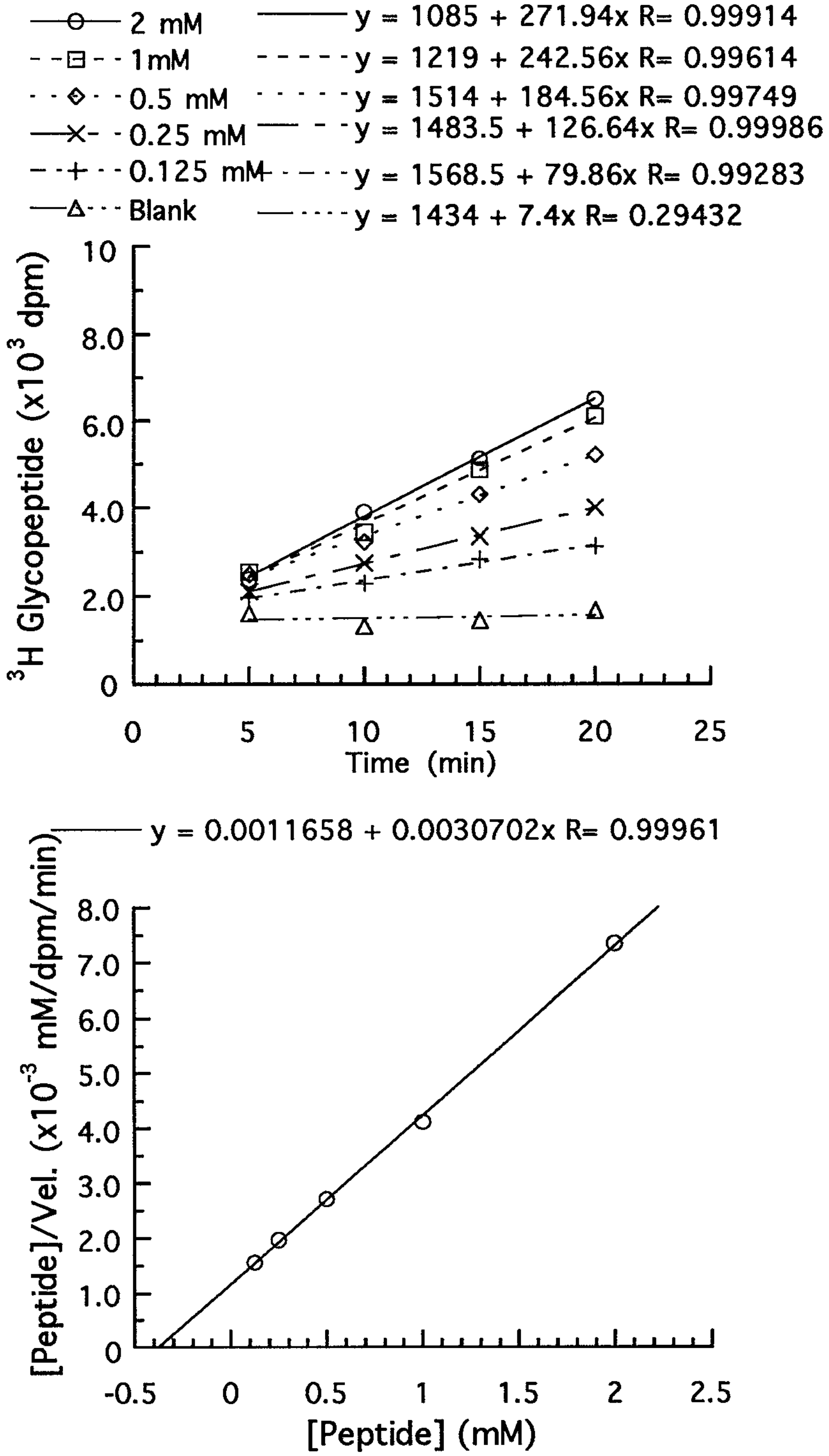
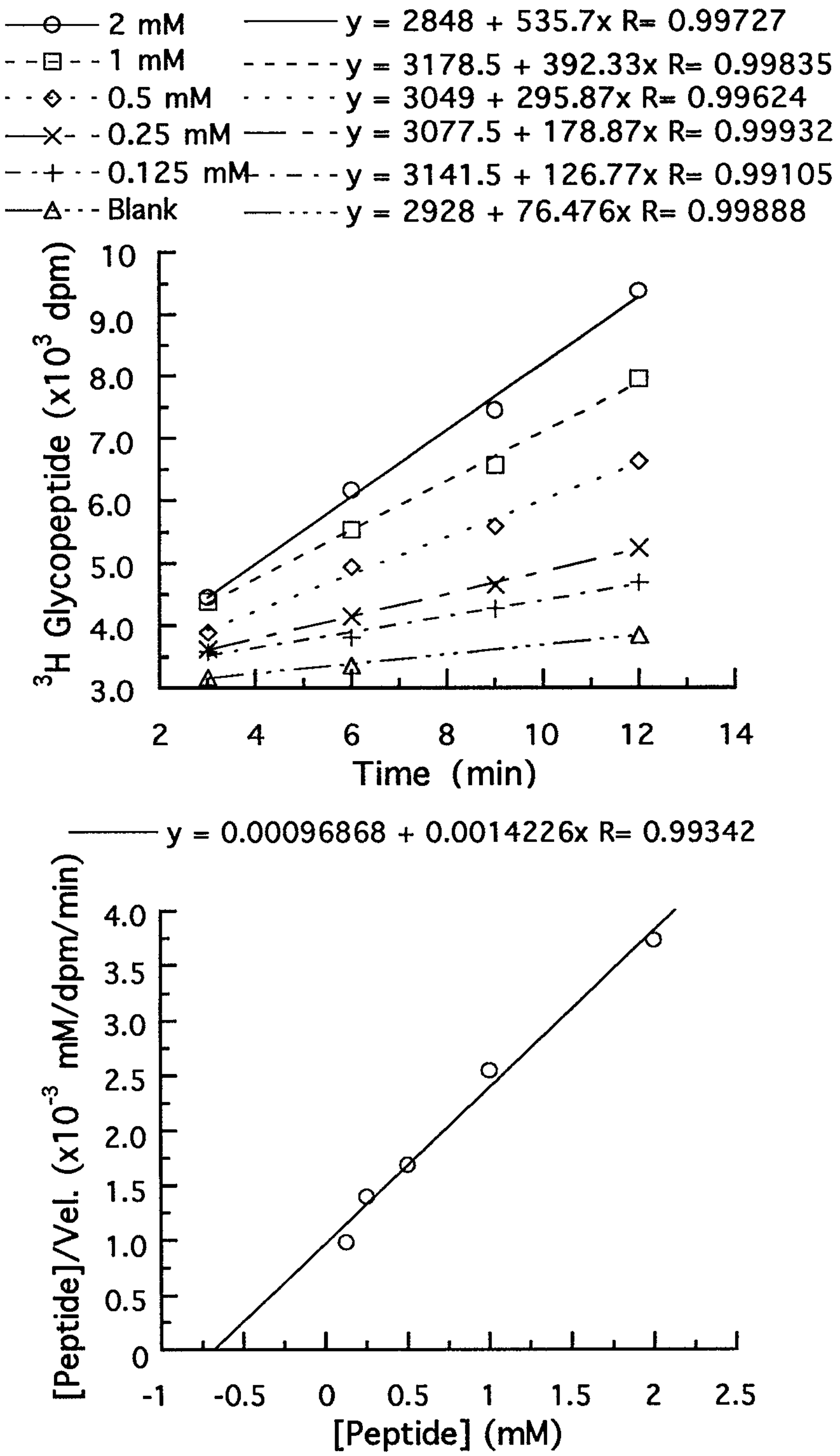
Kinetic Data for Bz-Asn(γS)-Leu-Thr-NHMe with Manganese



# Kinetic Data for Bz-Asn(γS)-Leu-Thr-NHMe with Iron



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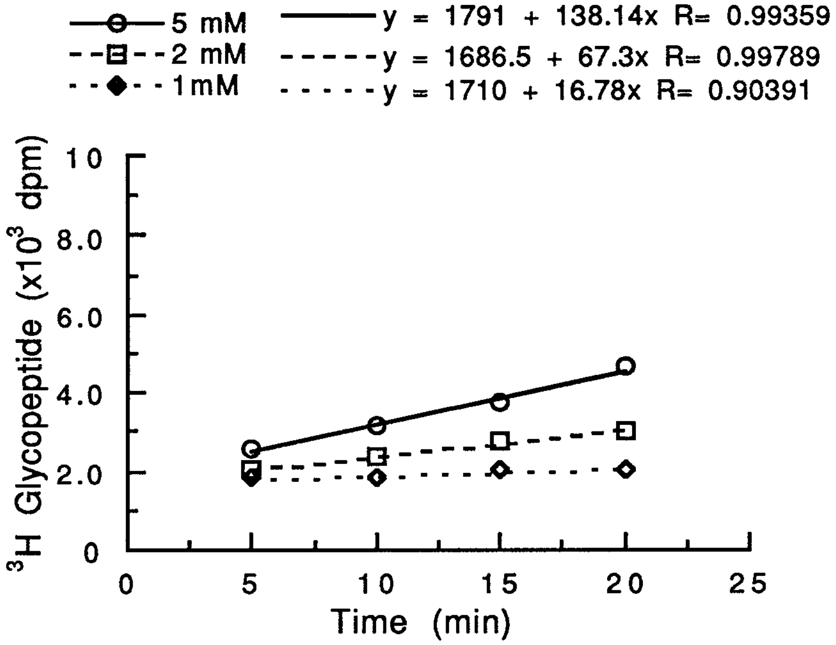
## Kinetic Data for Bz-Asn(γS)-Leu-Thr-NHMe with Magnesium



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## Kinetic Data for Bz-Asn(γS)-Leu-Thr-NHMe with Calcium