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Synthesis and Characterization of Rhenium and Technetium-99m Labeled Insulin

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A ^{99m}Tc-labeled insulin analogue was synthesized through a direct labeling method in which the $[^{99m}Tc(CO)_3]^+$ core was combined with a protected insulin derivative (**9**) bearing a M(I) chelate linked to the first amino acid of the B-chain (B1). Regioselective labeling was achieved by careful control over the pH and the reaction time. Following a TFA-anisole mediated deprotection step (decay-corrected yield of 30 ± 11%, $n = 4$), the identity of the final ^{99m}Tc-labeled product was confirmed by HPLC. Displacement of ^{125}I-insulin from the insulin receptor (IR) by the Re analogue **6** was similar to that of native insulin (17.8 nM vs 11.7 nM, respectively). The extent of autophosphorylation and Akt activation, as indicated by production of phospho-Akt (pAkt), showed no statistical difference between **6** and native insulin in both assays. These results support the use of the reported ^{99m}Tc-insulin derivative as a tracer for studying insulin biochemistry in vivo.

Introduction

Insulin is a polypeptide-based hormone that is featured prominently in energy homeostasis through its regulation of glucose uptake and influence over energy storing metabolites including lipids and proteins.[1] As a result of its central role in energy metabolism, abnormalities in insulin regulation are associated with a variety of diseases including diabetes, hypertension, and cancer.[2,3] Molecular imaging agents derived from human insulin for use in radioimaging studies offer a valuable, noninvasive means to study diseases that involve insulin disregulation in vivo.

A number of radiolabeled insulin analogues have been reported, including ^{125}I-insulin, which is widely used for in vitro insulin receptor (IR[a]) binding assays,[4−6] as well as ^{124}I-insulin[7,8] and ^{18}F-insulin[9,10] which have been used for positron emission tomography (PET) in a number of different studies including the determination of overexpression of insulin receptor in lymphoblastoid cells.[11] Insulin analogues labeled with isotopes suitable for use with single photon emission computed tomography (SPECT) have also been reported. ^{123}I-Insulin,[12,13] for instance, was used to study insulin biodistribution in diabetic subjects[11] and to detect human hepatocellular carcinoma.[14] Recently, a ^{67}Ga-insulin analogue has also been prepared and evaluated.[15−17] While these compounds have demonstrated the value of having an insulin-based probe, several of the existing agents suffer from poor stability in vivo and require complex production methods involving expensive isotopes, and some were reported with insufficient chemical and biological characterization to warrant future use in preclinical or clinical studies.

Despite the increasing interest in PET, SPECT remains the most predominant diagnostic nuclear medicine tool because of the lower capital and medical isotope costs compared to positron based methods. In particular, technetium-99m is an extremely attractive radioisotope for SPECT because of its low cost, widespread availability, and ideal nuclear properties ($E_\gamma = 140$ keV, $t_{1/2} = 6.02$ h).[18−21] Unlike ^{123}I and ^{67}Ga, technetium-99m is conveniently obtained from a generator and is now the most widely used isotope in nuclear medicine. As a result, a ^{99m}Tc-insulin analogue would be particularly attractive for use as a single photon emission computed tomography (SPECT) probe of insulin biochemistry. Awasthi et al. have reported the preparation of ^{99m}Tc-labeled insulin by treating the hormone directly with $^{99m}TcO_4^-$ in the presence of $SnCl_2$.[22] The labeling strategy resulted in reduction of the disulfide bonds and the formation of multiple labeled products, which limited the ability of the approach to generate a true insulin mimic.

An efficient strategy is reported here to synthesize a ^{99m}Tc-labeled insulin analogue as a tool to study the biochemistry and pharmacology of insulin in vivo. To avoid labeling at reactive amino acid residues and to ensure robust and covalent linkage of the metal to the targeting vector, a bifunctional chelate was employed. Our group has developed a series of M(I) chelators for Tc(I) and Re(I), which have been linked to a variety of biomolecules including peptides.[23,24] The ligands form robust metal complexes that are suitable for use in vivo. Building on this chemistry, a method was developed to label insulin in a regioselective manner at a site on the hormone that was designed to minimize the impact of the radiometal. On the

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[a] Abbreviations: IR, insulin receptor; PET, positron emission tomography; SPECT, single photon emission computed tomography; ELISA, enzyme-linked immunosorbant assay; BSA, bovine serum albumin; SEC, size exclusion chromatography.

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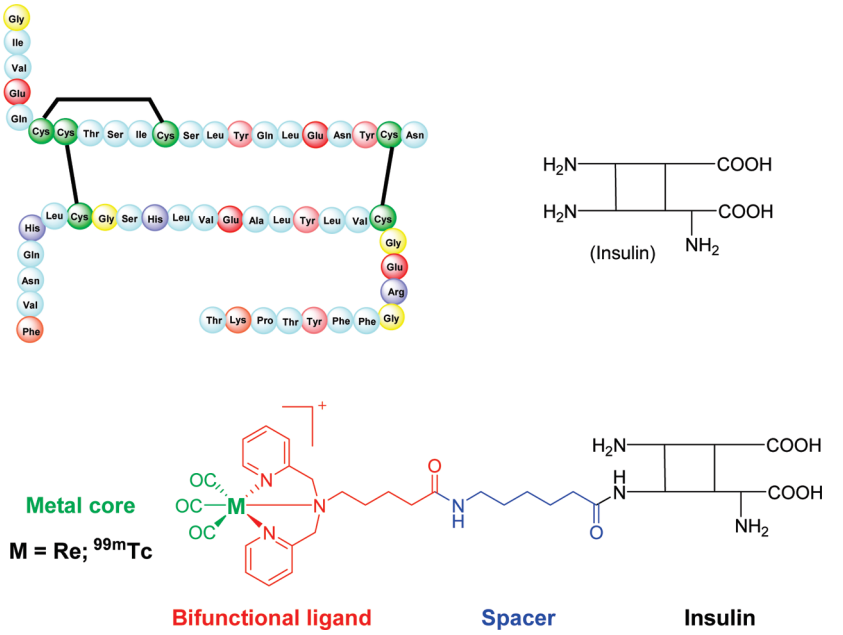
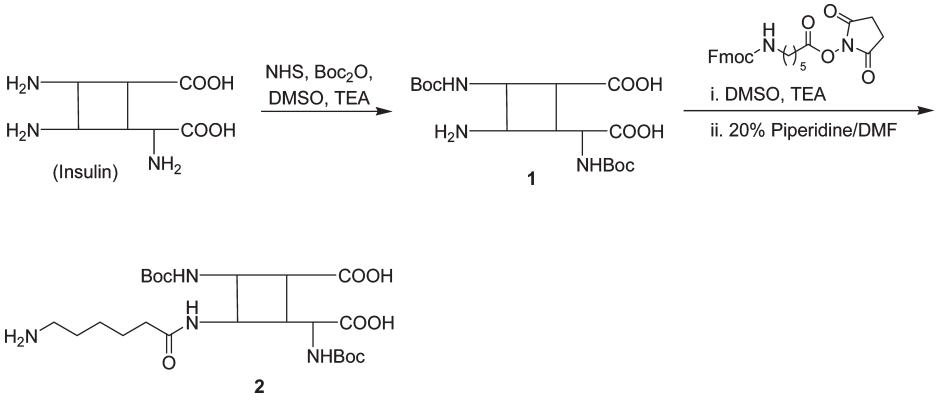


Figure 1. Human insulin and the target Tc/Re insulin conjugates.

Scheme 1. Synthesis of DBI (**1**) and AHx-DBI (**2**)



basis of literature precedents, the first amino acid of the B chain (PheB1) was selected as the linker site because it is insensitive to substitution even with bulky or lipophilic groups.[25,26]

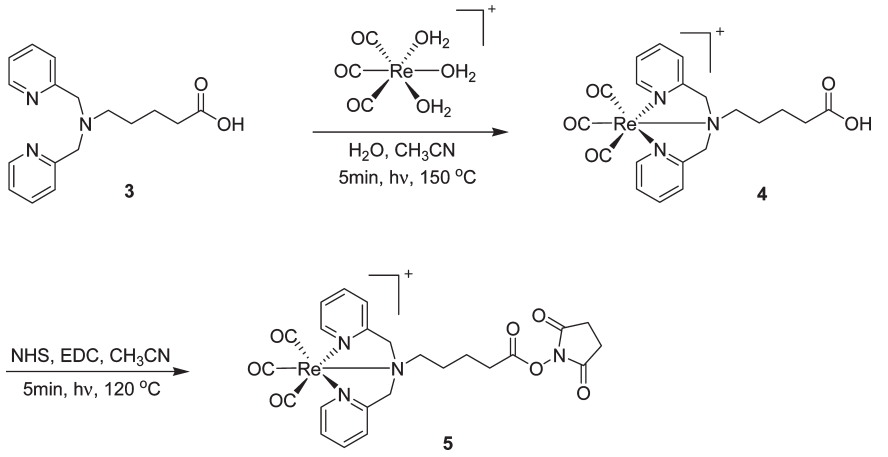
Results and Discussion

Synthesis and Characterization of B^1-[Re(CO)$_3${bis(2-pyridylmethyl)pentanoyl-6-aminohexanoyl)}]insulin (Re-BP-Pen-AHx-insulin, 6). The first step in making a viable ^{99m}Tc-insulin analogue was to isolate the Re analogue of the target and determine if the chosen synthetic route, site of conjugation, and nature of the linker group and chelate resulted in any significant alteration of the biochemical properties of the hormone. Re was used as a surrogate, since there are no stable isotopes of technetium, which is a widely accepted approach as the metals are congeners and therefore form isostructural products, particularly in the oxidation state used here. The synthetic route chosen paralleled that previously reported by Shai and co-workers[27] and our group[10] in which insulin was modified at the amino terminus of the PheB1 amino acid residue with an ^{18}F prosthetic group and a spacer. This method takes advantage of the differing reactivity of the three primary amines present within the structure of insulin, where the more reactive amines LysB29 and GlyA1 can be protected with Boc groups, leaving the PheB1 amine free for further derivatization.[27,28]

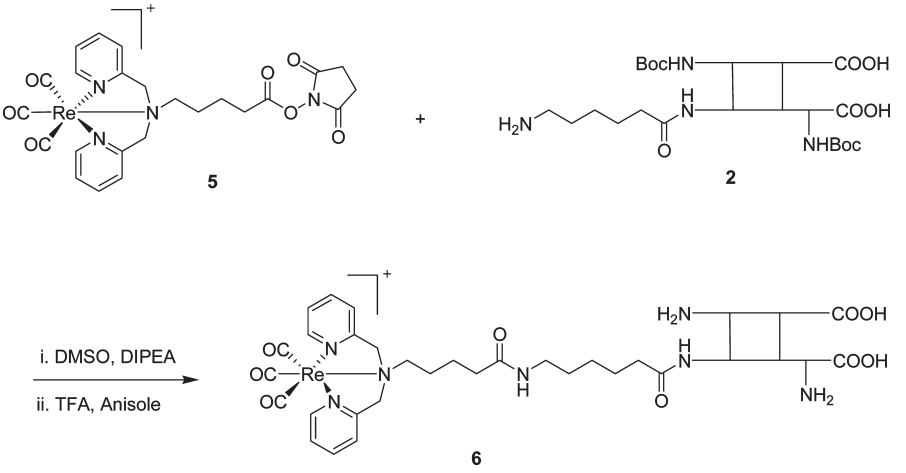
We have previously found that the addition of a short aminohexanoic acid spacer at the PheB1 site was beneficial for labeling at the tracer level with ^{18}F,[10] and as such, we chose to maintain this strategy for labeling with Re and ^{99m}Tc. Our initial step toward synthesizing the target compound was to convert insulin to the diBoc derivative **1**, followed by conjugation to the aminohexanoic acid spacer via its active ester, to give **2**, as previously reported (Scheme 1).[10]

To avoid labeling at reactive amino acid residues and to ensure robust and covalent linkage of the metal to the targeting vector, a bifunctional chelate was employed. The chelate of choice was described by Banerjee et al.[29] and consists of the bispyridyl chelate (Figure 1) that forms a highly stable complex with the M(CO$_3$)$^+$ cores (M = Re, ^{99m}Tc). Succinimidyl [Re(CO)$_3$(bis(2-pyridylmethyl)pentanoate)] **5** was used to prepare the rhenium standard, as it is the metal analogue of succinimidyl 4-fluorobenzoate which we used successfully to label insulin with ^{18}F and ^{19}F. Compound **5** was prepared as reported in the literature in good yields (Scheme 2).[30] The active ester was used immediately upon isolation because it rapidly hydrolyzes, which was confirmed by observing changes in the

Scheme 2. Synthesis of Active Ester **5**



Scheme 3. Synthesis of Re-BP-Pen-AHx-insulin (**6**)



^{1}H NMR over time. In addition, samples of the active esters reported here were difficult to obtain completely dry and were often isolated with trace amounts of residual solvent.

The bioconjugate Re-BP-Pen-AHx-insulin (**6**) was produced by coupling the Boc-protected insulin precursor **2** with the active ester **5**, in DMSO containing 5% *N*,*N*-diisopropylethylamine (DIPEA), followed by isolation by precipitation and centrifugation (Scheme 3). The protecting groups were removed using TFA containing 5% anisole,[31] and the desired material was isolated by preparative reversed-phase HPLC. The overall yield of Re-BP-Pen-AHx-insulin was 46%, and the purity was greater than 95% as determined by HPLC. Electrospray mass spectrometry was used to determine the identity of compound **6**, which displayed a spectrum of multiply charged ions at *m*/*z* 2158.1 [M + 3H$^+$]/3, 1618.8 [M + 4H$^+$]/4, and 1295.2 [M + 5H$^+$]/5, which corresponded to the calculated molecular mass of the parent (6471.3 g/mol).

A more convergent synthetic route to **6** was developed subsequently, wherein diBoc-insulin **1** was conjugated to compound **8**, which possesses the chelate and the desired aminohexanoic acid linker (Scheme 4). The active ester **8** was prepared from acid **7**, which in turn was obtained by the reaction of **5** with commercially available 6-aminocaproic acid. While the overall yields were comparable in both approaches, the convergent route was found to be more convenient because it had fewer steps that required purification by preparative HPLC.

To verify the precise site of derivatization, fragments of compound **6** were generated by reduction followed by digestion and analyzed by LC−MS. The sample was first treated with dithiothreitol to disrupt the interchain disulfide bonds, yielding separate A and B chains. LC−MS (ESI(+)) analysis, for which the data are listed in Table 1, had three major peaks at 89.2, 98.0, and 105.9 min. The peak at 89.2 min exhibited a *m*/*z* value of 1190.6, which corresponds to the free unmodified A-chain. The peak at 98.0 min displayed *m*/*z* values of 1618.9 and 1295.3 corresponding to the [M + 4H$^+$]/4 and [M + 5H$^+$]/5 ions of the intact insulin bioconjugate. The final peak at 105.9 min had *m*/*z* values of 1024.0 and 1364.9, which corresponded to the [M + 4H$^+$]/4 and [M + 3H$^+$]/3 ions for the modified B-chain. These results confirm that the rhenium pendant group modification was not attached at the glycine-A1 site.

To verify the B-chain modification site, the sample was treated with endoproteinase Glu-C, which cleaves peptides at the carbonyl side of glutamic acid residues. LC−MS analysis of the peptide digest was performed, and the results are summarized in Table 2. A peak at 66.7 min gave rise to a signal at *m*/*z* 417.4, which corresponds to the GIVE [M + H]$^+$ fragment from the A-chain of insulin. A peak at 78.9 produced a signal at *m*/*z* 1116.7, which was consistent with

Scheme 4. Alternative Synthesis of Re-BP-Pen-AHx-insulin (**6**)

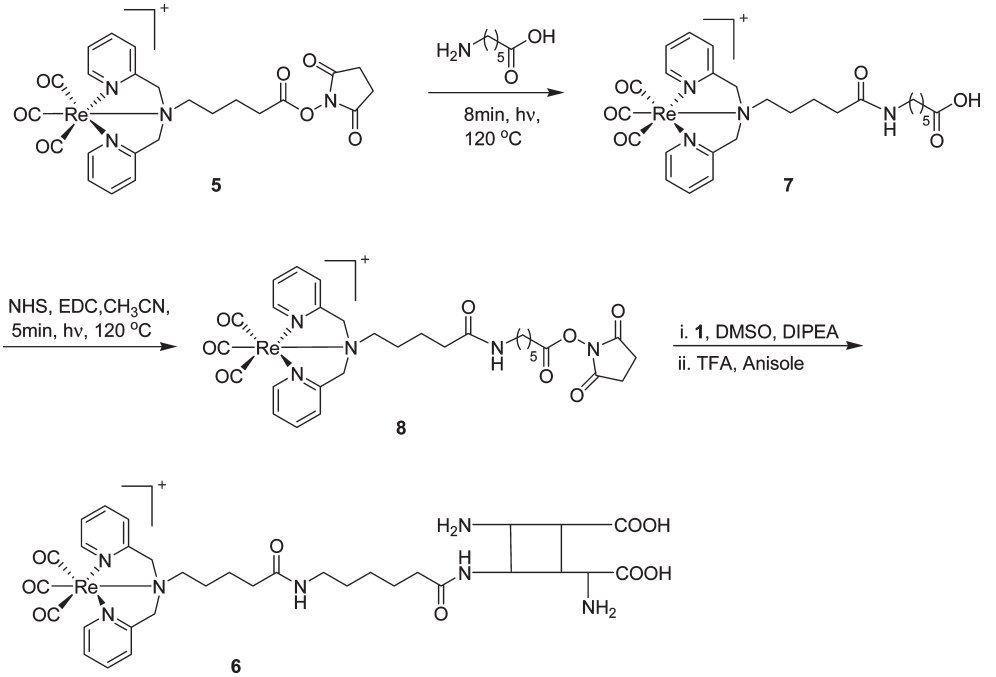


Table 1. LC-MS (ESI+) Data for **6** after Treatment with Dithiothreitol (DTT)

HPLC t_R[a](min)	*m*/*z* found (calcd)	molecular ion	fragment
89.2	1190.6 (1192.9)	$[M + 2H^+]/2$	A-chain
98.0	1618.9 (1618.8)	$[M + 4H^+]/4$	intact **6**
	1295.3 (1295.2)	$[M + 5H^+]/5$	
105.9	1364.9 (1365.9)	$[M + 3H^+]/3$	modified B-chain of **6**
	1024.0 (1024.7)	$[M + 4H^+]/4$	

[a] Column C_{18} Beckman Ultrasphere (150 mm × 4.6 mm, 5 μm particle). HPLC elution conditions: mobile phase A, water with 0.1% TFA; mobile phase B, acetonitrile with 0.05% TFA; gradient 99:1 (A/B) for 10 min, 99:1 (A/B) to 11:89 (A/B) over 140 min, 11:89 (A/B) to 1:99 (A/B) for 3 min, 1:99 (A/B) for 17 min; flow rate 0.1 mL/min. ESI+ MS scanned from *m*/*z* 0 to *m*/*z* 2400.

the molecular ion $[M + H]^+$ of the RGFFYTPKT fragment of the B-Chain. A peak at 95.5 min exhibited a *m*/*z* of 1129.4, which corresponded to the TFA adduct of the N-terminally modified FVNQHLCGSHLVE B-chain fragment $[M + TFA + 2H^+]/2$. Together, these data confirm that the site of conjugation was at the amino terminus of the Phe-B1 site and not at either the Lys-B29 or Gly-A1 primary amines.

In Vitro Testing of Re-BP-Pen-AHx-insulin. To evaluate the biological properties of compound **6**, three in vitro assays were performed to probe the vital points within the insulin-signaling cascade. The first assay was performed to determine the ability of **6** to bind to the insulin receptor by assessing the displacement of ^{125}I-insulin from the IR by both **6** and native insulin. The behavior of **6** bound to the IR was found to be markedly similar to that of native insulin (Figure 2), giving IC_{50} values of 17.8 and 11.7 nM for the modified and native insulin, respectively. Although the binding properties were similar for the native and modified insulin, further evidence to confirm that **6** retained its core physiological functions was obtained by two functional in vitro assays. The insulin receptor autophosphorylation was assessed through the use of an enzyme-linked immunosorbant assay (ELISA). It was found that there was no significant difference observed in the extent of autophosphorylation induced by **6** compared to unmodified human insulin (Figure 2). The calculated EC_{50} for autophosphorylation by unmodified human insulin was 2.48 nM, whereas that for Re-BP-Pen-AHx-insulin was 4.22 nM. Finally, to probe the downstream signaling resulting from insulin binding, a second ELISA experiment was performed to monitor stimulation of Akt phosphorylation (S473). It was found that the response to Re-BP-Pen-AHx-insulin binding was not significantly different from that induced by unmodified human insulin (Figure 2). The calculated EC_{50} for stimulation of Akt1 phosphorylation was 0.13 nM for both human insulin and compound **6**.

The results of the biochemical assays showed that chemical modification at the PheB1 site had minimal impact on the binding of insulin to the IR and supports the use of the isostructural ^{99m}Tc analogue as a mimic of insulin for in vivo studies.

Radiochemistry. Production of ^{99m}Tc-based radiopharmaceuticals is typically performed using instant kits. As a result, the preparation of the ^{99m}Tc analogue of **6** followed a direct labeling strategy that could in future be adapted to an instant kit formulation. Compound **9**, prepared in an analogous manner to its rhenium standard, (Schemes 5 and 6) was selected as an appropriate precursor, as it would require only one additional synthetic step (i.e., deprotection) to form the desired ^{99m}Tc-BP-Pen-AHx-insulin product (**10**) following addition of the $[^{99m}Tc(CO)_3]^+$ core (Scheme 7). We chose to keep the Boc protecting groups present on the insulin precursor to reduce the likelihood of nonselective coordination of the metal core to the relatively nucleophilic primary amines on GlyA1 and LysB29. Using this strategy, we were able to regioselectively obtain the desired ^{99m}Tc insulin without degradation of insulin or generation of undesirable side products, unlike the previously reported methods.

Table 2. LC-MS (ESI+) Data of DTT Treated **6** after Digestion with Endoproteinase Glu-C

HPLC t_R[a] (min)	m/z found (calcd)	molecular ion	fragment
66.7	417.4 (417.4)	$[M + H^+]$	GIVE (A-chain)
78.9	1116.7 (1116.3)	$[M + H^+]$	RGFFYTPKT (B-chain)
95.5	1129.4 (1129.3)	$[M + TFA + 2H^+]/2$	Re-BP-Ahx-FVNQHLCGSHLVE (B-chain)

[a] Column C_{18} Beckman Ultrasphere (150 mm × 4.6 mm, 5 μm particle). HPLC elution conditions: mobile phase A, water with 0.1% TFA; mobile phase B, acetonitrile with 0.05% TFA; gradient 99:1 (A/B) for 10 min, 99:1 (A/B) to 11:89 (A/B) over 140 min, 11:89 (A/B) to 1:99 (A/B) for 3 min, 1:99 (A/B) for 17 min; flow rate 0.1 mL/min. ESI+ MS scanned from m/z 0 to m/z 2400.

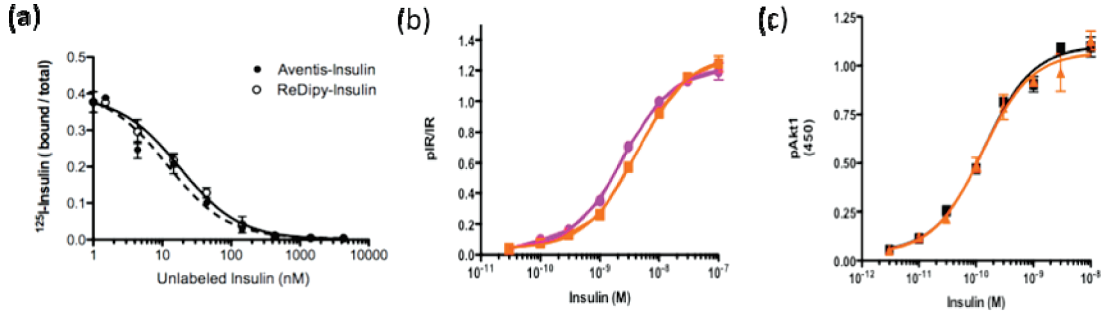
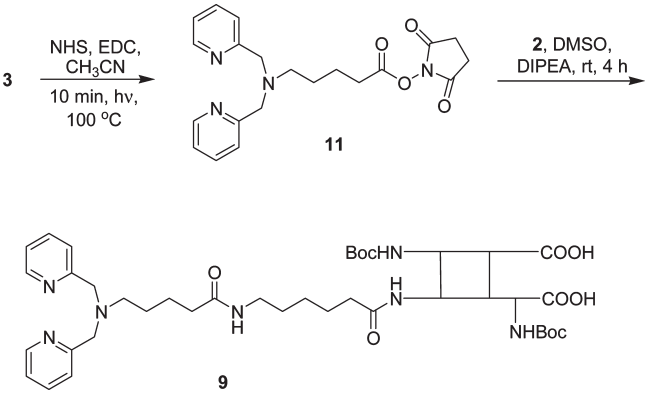


Figure 2. (a) Displacement assay of ^{125}I-insulin with **6** (open circles) and native insulin. (b) Comparison of human insulin (circles) and **6** (squares) in insulin receptor autophosphorylation ELISA. (c) Comparison of human insulin (squares) and **6** (triangles) in Akt phosphorylation ELISA.

Scheme 5. Synthesis of BP-Pen-AHx-DBI (**9**)



The technetium precursor, $[^{99m}Tc(CO)_3(OH_2)_3]^+$, was formed from $^{99m}TcO_4^-$ using our previously reported microwave methodology.[30] After the solution was cooled to room temperature, the pH of the solution containing the $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was varied between 5.0 and 7.5 using HCl. Compound **9** (2 mg, 312 nmol) was added to a mixture of CH_3CN and H_2O (1:2) and the solution stirred at various temperatures and times. The reaction progress was monitored by HPLC. The pH of the reaction mixture was found to be an important factor in determining the efficiency of labeling. The optimal pH identified was 6.5, which minimized the amount of nonspecific labeling. This is likely due to the protonation of the histidine residues on insulin, which are known to be good donors for Tc(I).[32] A combination of pH 6.5, a temperature of 45 °C, and a reaction time of 90 min minimized the amount of nonspecific labeling (Figure 3a) and ultimately proved to be the most effective of the labeling conditions tested.

Following labeling, the reaction mixture was evaporated to dryness at 38 °C using a Biotage V10 solvent evaporator and then the Boc groups were cleaved by dissolution of the dried mixture in TFA containing 5% anisole. The deprotected mixture was then purified by semipreparative reverse-phase HPLC. The major product was collected, dried using the V10 evaporator, and resuspended in buffered saline containing 1% (w/w) bovine serum albumin (BSA). The final purified product **10** was obtained, after a total reaction time of 4 h, in excellent radiochemical purity and 30% decay-corrected yield (30 ± 11%, n = 4), and its retention time corresponded to the elution of the reference standard **6**. This labeling strategy was amenable to producing sufficient quantities of labeled product (407 MBq, 11 mCi) from modest amounts of $^{99m}TcO_4^-$ (1.4 GBq, 38 mCi) for preclinical studies. Larger scale production runs have also been successfully completed using 11.1 GBq (300 mCi) of $^{99m}TcO_4^-$ generating 1.78 GBq (48 mCi, nondecay corrected) of **10** requiring slightly over 1 half-life (7 h) in which to isolate and reconstitute the product.

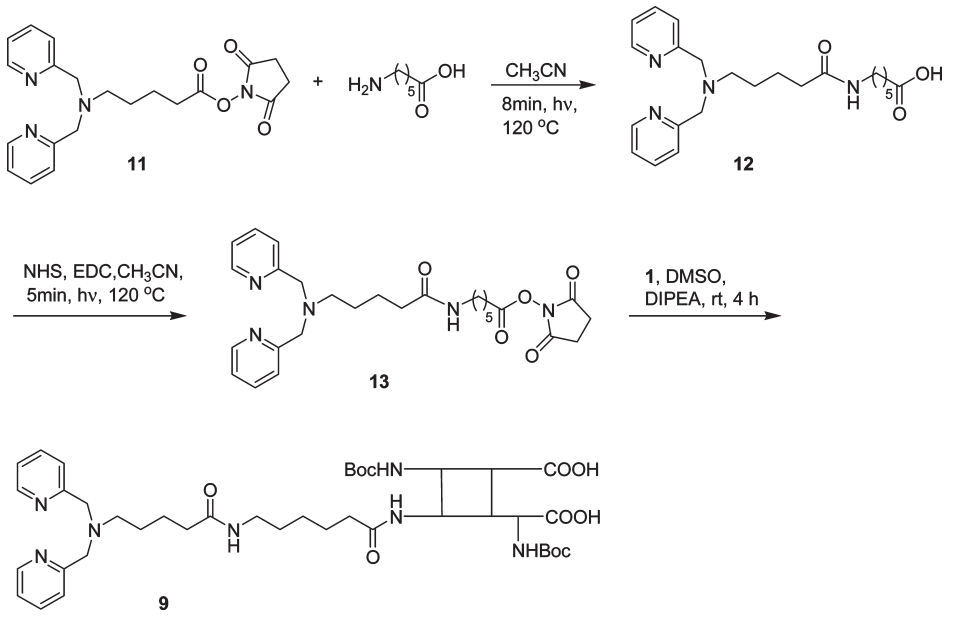
Conclusions

A regioselective method for labeling insulin with ^{99m}Tc was developed. The radioactive product was characterized by comparison to the nonradioactive and fully characterized reference standard **6**. In a series of screening assays, the rhenium analogue retained the biological characteristics of native insulin, which supports the use of the ^{99m}Tc analogue as a tracer for studying insulin biodistribution and biochemistry in vivo. The probe is currently being evaluated in a series of biodistribution and SPECT/CT studies in different preclinical disease models where the extent of specific and nonspecific binding will be assessed.

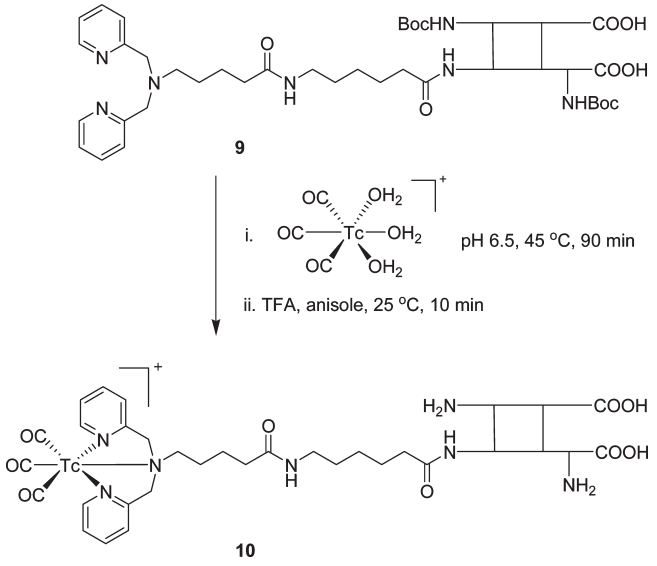
Experimental Section

Materials and Instrumentation. Reagents and solvents were purchased from Aldrich Inc., NovaBiochem Inc., or Fluka Inc. and were used without further purification. Human insulin was obtained from Aventis Inc., and ^{125}I-insulin was obtained from Amersham Inc. Size-exclusion chromatography (SEC) was performed using HiTrap desalting cartridges (GE Healthcare). SEC cartridges were activated with 100 mM NH_4HCO_3 (20 mL) prior to use. Following the desalting, the cartridges were washed with the NH_4HCO_3 buffer (20 mL), H_2O (20 mL), and 80/20 (v/v) H_2O/EtOH (20 mL). Solid-phase extraction C18 SepPak cartridges (Waters) were activated with EtOH (10 mL) followed by H_2O (10 mL).

Scheme 6. Alternative Route for the Synthesis of BP-Pen-AHx-DBI (**9**)



Scheme 7. Synthesis of ^{99m}Tc-BP-Pen-AHx-insulin (**10**)



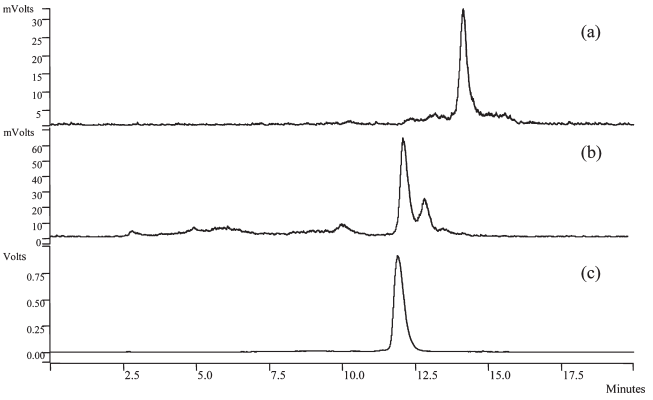


Figure 3. Radio-HPLC chromatograms of (a) crude reaction mixture following the reaction of $[^{99m}Tc(CO_3)(OH_2)_3]^+$ with **9**, (b) crude reaction mixture following deprotection of ^{99m}Tc-BP-Pen-Ahx-DBI with TFA and anisole, and (c) purified **10**.

Both nonradioactive and radioactive analytical HPLC experiments were performed using Varian ProStar HPLC systems fitted with a 330 PDA multiwavelength detector, a 230 solvent delivery module, and a Beckman Ultrasphere C_{18} column (4.6 mm × 100 mm, 300 Å, 5 μm) or Phenomenex Gemini C_{18} column (4.6 mm × 150 mm, 300 Å, 5 μm). For analytical experiments, mobile phases were as follows: (A) H_2O + 0.1% TFA; (B) CH_3CN + 0.05% TFA. A gradient profile of 75/25 to 20/80 A/B (v/v) over 20 min, 20/80 A/B to 0/100 A/B over 5 min, followed by an isocratic wash of 0/100 A/B over 5 min (method A) was used. Absorbance data were collected from 210 to 400 nm where a wavelength of 254 nm was used to monitor the elution profiles.

For preparative and semipreparative HPLC experiments, a Varian ProStar preparative HPLC system, which consisted of a model 320 detector, a model 215 solvent delivery module, and a Microsorb Dynamax C_{18} column (41.4 mm × 250 mm, 300 Å, 8 μm) for preparative experiments and a Phenomenex Gemini C_{18} column (9.2 mm × 500 mm, 300 Å, 5 μm) for semipreparative experiments, was used. All purification runs were performed using H_2O + 0.1% TFA (mobile phase A) and CH_3CN + 0.05% TFA (mobile phase B). Absorbance was monitored at a wavelength of 254 nm. For purification of **1** and **2**, the preparative C18 column was used and the elution protocol consisted of a gradient profile of 75/25 to 30/70 A/B (v/v) over 30 min, 30/70 A/B to 0/100 A/B over 5 min, followed by an isocratic wash of 0/100 A/B for 5 min, all at a flow rate of 45 mL/min (method B). For purification of **6**, **9**, and **10**, the semipreparative C_{18} column was used and the elution protocol consisted of a gradient profile of 75/25 to 20/80 A/B (v/v) over 20 min, 20/80 A/B to 0/100 A/B over 5 min, followed by an isocratic wash of 0/100 A/B over 5 min, all at a flow rate of 4 mL/min (method C).

ESI-MS experiments and MALDI-MS experiments were carried out on Micromass Quattro Ultima and TOF Spec2E instruments, respectively. Prior to each MALDI TOF analysis, a calibration standard was run that consisted of a mixture of 1 fmol/μL substance P, 2 pmol/μL resin substrate tetradecapeptide, 2 pmol/μL adrenocorticotropic hormone fragment 18−39, and 10 pmol/μL cytochrome *c*. This was done in the positive ion reflection mode at 20 keV. NMR spectra were recorded using either a Bruker AV 600 MHz or DRX 500 MHz instrument. Chemical shifts (δ) are reported in ppm. Coupling constants (*J*) are reported in hertz (Hz). NMR spectra were referenced to the

residual proton peaks in the deuterated solvents ($CHCl_3$, 7.26 ppm; CH_3OH, 3.31 ppm; CH_3CN, 1.94 ppm) for ^1H NMR and to the carbon signals of the deuterated solvents ($CDCl_3$, 77.16 ppm; CD_3OD, 49.0 ppm; CD_3CN, 118.26 ppm) for ^{13}C NMR spectra.

Synthetic Procedures. A^1,B^{29}-Di-(*tert*-butyloxycarbonyl)insulin (DBI, 1) and B^1-(6-Aminohexanoyl)-A^1,B^{29}-di-(*tert*-butyloxycarbonyl)insulin (AHx-DBI, 2). Compounds **1** and **2** were synthesized by a previously reported procedure.[10] Following the reaction workup, the compounds were dissolved in 75/25 (v/v) H_2O/CH_3CN containing 0.1% TFA and purified by preparative HPLC (method B). Fractions collected were then concentrated in vacuo to remove most of the CH_3CN and then lyophilized overnight. The resulting solid was then desalted using SEC cartridges by elution with a 100 mM NH_4HCO_3 buffer. The fractions were again concentrated in vacuo, followed by lyophilization overnight to yield colorless solids. The characterization data matched the literature values, and the yields were comparable (65−70%). The purity for both compounds was >95% as determined by analytical HPLC (method A).

Bis(2-pyridylmethyl)pentanoic Acid (BP-Pen) (3). 2-Pyridine carboxaldehyde (2.23 g, 20.8 mmol), 5-aminopentanoic acid (0.97 g, 8.3 mmol), and sodium triacetoxyborohydride (4.5 g, 21.3 mmol) were combined and stirred at room temperature in 30 mL of dichloroethane for 18 h and then concentrated to dryness using a rotary evaporator. The residue was dissolved in 50 mL of water and extracted with 25 mL of ethyl acetate three times, and then the aqueous phase was concentrated to dryness using a rotary evaporator. The crude residue was purified by silica gel chromatography using 10/90 $MeOH/CH_2Cl_2$ (v/v) as the eluent. The product (1.5 g, 54%) was a yellow viscous liquid, and its characterization data matched that data reported in the literature.[33]

$Re(CO)_3$-BP-Pen (4). A solution of $[Re(CO)_3(OH_2)_3]Br$[34] (1.04 g, 2.54 mmol) and **3** (462 mg, 1.54 mmol) in 3 mL of H_2O and 1 mL of CH_3CN was combined in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the vial was crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor at 150 °C for 5 min. The product was isolated by silica gel chromatography, and the separation was performed using 10/90 $MeOH/CH_2Cl_2$ (v/v) as the eluent. The product was an off-white solid (0.55 g, 62%), and its characterization data matched the data reported in the literature.[30]

Succinimidyl [$Re(CO)_3$-BP-Pen] (5). A solution of **4** (180 mg, 0.28 mmol), *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide (EDC) (266 mg, 1.38 mmol), and *N*-hydroxysuccinimide (NHS) (160 mg, 1.39 mmol) in 5 mL of acetonitrile was combined in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the vial was crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor at 120 °C for 5 min. The crude reaction mixture was concentrated using a rotary evaporator, dissolved in CH_2Cl_2, and extracted with water (25 mL × 2), and the organic phase was concentrated to dryness. The crude sample was then purified using a Biotage SP1 purification system affixed with a disposable silica column, and separation was performed using a solvent gradient 3/97 (v/v) $MeOH/CH_2Cl_2$ to 20/80 $MeOH/CH_2Cl_2$ (v/v). The product was isolated as an orange oil (192 mg, 91%), and its characterization data matched the data reported in the literature.[30]

$Re(CO)_3$-BP-Pen-AHx (7). Method A. A solution of **5** (167 mg, 0.25 mmol) in 5 mL of CH_3CN and 6-aminocaproic acid (170 mg, 1.30 mmol) was combined in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the vial crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor at 120 °C for 8 min. The precipitate (unreacted aminocaproic acid) was removed by filtration, and the crude reaction mixture was concentrated using a rotary evaporator. The sample was then purified using silica gel chromatography and a solvent gradient 5/95 (v/v) $MeOH/CH_2Cl_2$ to 20/80 $MeOH/CH_2Cl_2$ (v/v). The product was isolated as an off-white solid (104 mg, 61%).

Method B. To a solution of **12** (164 mg, 0.4 mmol) in 3.8 mL of water and 1.2 mL of acetonitrile in a 2−5 mL Emery's process containing a magnetic stir bar was added $[Re(CO)_3(OH_2)_3]Br$ (279 mg, 0.69 mmol), and the vial crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor at 150 °C for 5 min and the solution concentrated to dryness using a rotary evaporator. The residue was then purified using silica gel chromatography, and separation was performed using a solvent gradient 10/90 (v/v) $MeOH/CH_2Cl_2$ to 20/80 $MeOH/CH_2Cl_2$ (v/v). The product was obtained as an off-white solid (265 mg, 98%). Mp 103 °C. R_f (CH_2Cl_2/MeOH, 90:10, v/v): 0.35. High resolution ES MS(+) calcd for $C_{26}H_{32}O_6N_4Re$, *m*/*z* 681.1841 and 683.1880 for ^{185}Re and ^{187}Re, found 681.1802 and 683.1841, respectively. ^1H NMR δ (500.13 MHz, CD_3CN): 8.78 (d, J = 5.0, 2H, PyH), 7.90 (t, J = 8.0, 2H, PyH), 7.53 (d, J = 8.0, 2H, PyH), 7.31 (t, J = 6.5, 2H, PyH), 6.84 (m, 1H, NHCO), 4.91 (d, J = 16.5, 2H, $PyCH_2$), 4.69 (d, J = 16.5, 2H, $PyCH_2$), 3.81 (t, J = 8.0, 2H, NCH_2), 3.17 (q, J = 6.5, 2H, $CONHCH_2$), 2.29 (m, 4H, CH_2CO), 1.92 (m, 2H, CH_2), 1.70 (m, 2H, CH_2), 1.61 (m, 2H, CH_2), 1.53 (m, 2H, CH_2), 1.35 (m, 2H, CH_2). ^{13}C NMR δ (125.76 MHz, CD_3CN): 197.2, 196.5, 175.8, 173.1, 161.6, 153.0, 141.3, 126.5, 124.6, 71.1, 68.4, 39.6, 36.2, 34.8, 29.9, 27.1, 25.4, 25.1, 23.5.

Succinimidyl [$Re(CO)_3$-BP-Pen-AHx] (8). A solution containing **7** (240 mg, 0.35 mmol), EDC (337 mg, 1.76 mmol), and NHS (202 mg, 1.76 mmol) in 4 mL of acetonitrile was placed in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the vial was crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor for 5 min at 120 °C. The crude reaction mixture was concentrated using a rotary evaporator, the residue dissolved in CH_2Cl_2 which was then extracted with water (30 mL × 2), and the organic phase concentrated to dryness. The sample was then purified by silica gel chromatography using 10/90 $MeOH/CH_2Cl_2$ (v/v). The product was isolated as a pale-yellow oil (245 mg, 89%). R_f (CH_2Cl_2/MeOH, 90:10, v/v): 0.48. High resolution ES MS(+) calcd for $C_{30}H_{35}O_8N_5Re$, *m*/*z* 778.2038 and 780.2043 for ^{185}Re and ^{187}Re, found 778.2029 and 780.2036, respectively. ^1H NMR δ (500.13 MHz, CD_3OD): 8.85 (d, J = 5.0, 2H, PyH), 7.94 (t, J = 8.0, 2H, PyH), 7.57 (d, J = 8.0, 2H, PyH), 7.37 (t, J = 6.5, 2H, PyH), 4.86 (dd, J = 16.5 and 12.0, 4H, $PyCH_2'$), 3.83 (m, 2H, NCH_2), 3.22 (t, J = 7.0, 2H, $CONHCH_2$), 2.83 (s, 4H succinimidyl-CH_2), 2.65 (m, 2H, CH_2CO), 2.36 (m, 2H, CH_2), 1.98 (m, 2H, CH_2), 1.76 (m, 4H, CH_2), 1.53 (m, 4H, CH_2). ^{13}C NMR δ (125.76 MHz, CD_3OD): 197.2, 175.3, 171.8, 170.2, 162.1, 153.1, 141.6, 126.9, 124.6, 71.4, 68.8, 54.8, 40.1, 36.3, 31.5, 29.8, 27.0, 26.5, 26.3, 25.8.

Re-BP-Pen-AHx-insulin (6). Step 1. Method A. A solution of B^1-(6-aminohexanoyl)-A^1,B^{29}-di-(*tert*-butyloxycarbonyl)insulin (AHx-DBI, **2**) (10.0 mg, 1.3 μmol) and **5** (12.2 mg, 16.3 μmol) in DMSO (1.25 mL) containing *N*,*N*-diisopropylethylamine (DIPEA) (25 μL) was stirred for 90 min at room temperature. The reaction mixture was then transferred to a centrifuge vial containing 15 mL of CH_3CN. Then Et_2O was added slowly until a white precipitate formed. The precipitate was isolated by centrifugation at 3500 rpm for 30 min at 5 °C. The resulting pellet was washed twice with 100% CH_3CN and reisolated by centrifugation. The pellet was then dissolved in 75/25 H_2O/CH_3CN containing 0.1% TFA and purified by preparative reverse-phase HPLC. The desired fractions were collected and concentrated by rotary evaporation (water bath 37 °C) to remove most of the CH_3CN. Following lyophilization, Re-BP-Pen-AHx-DBI (7.1 mg, 65%) was obtained as a white powder. Analytical HPLC t_R = 9.8 min (Beckman Ultrasphere C_{18} column 100 mm × 4.6 mm, method A). ES MS(+) calcd 1335.2 [M + $5H^+$]/5, 1668.8 [M + $4H^+$]/4; found 1335.4 and 1668.9, respectively.

Method B. A solution of A^1,B^{29}-di-(*tert*-butyloxycarbonyl)-insulin (DBI, **1**) (10.0 mg, 1.67 μmol) and **8** (13 mg, 16.7 μmol) in DMSO (0.4 mL) containing DIPEA (5%) was stirred for 2 h at

room temperature. The reaction mixture was then transferred to a centrifuge vial containing 15 mL of CH_3CN, and Et_2O was added slowly until a white precipitate formed. The precipitate was isolated by centrifugation at 3500 rpm for 30 min at 5 °C, then washed twice with 100% CH_3CN and isolated by centrifugation. The purification procedure was the same as in method A. Following lyophilization, Re-BP-Pen-AHx-DBI (4.2 mg, 38%) was obtained as a white powder.

Step 2. Re-BP-Pen-AHx-DBI (5.0 mg) was dissolved in 500 μL of TFA containing 5% anisole (v/v) and allowed to react at room temperature for 30 min. The deprotected product was then precipitated in 25 mL of Et_2O and isolated by centrifugation at 3500 rpm for 30 min at 5 °C. The precipitate was washed twice with 100% CH_3CN followed by centrifugation. The solid was then dissolved in 75/25 H_2O/CH_3CN containing 0.1% TFA and purified by preparative reverse-phase HPLC. The desired fractions were collected and concentrated by rotary evaporation (water bath 37 °C) to remove most of the CH_3CN. Following lyophilization, Re-BP-Pen-AHx-insulin (3.4 mg, 71%) was obtained as a white powder. Analytical HPLC (method A) t_R = 9.2 min (Phenomenex Gemini C_{18} 150 mm × 4.6 mm). ES MS(+) calcd 1079.5 [M + $6H^+$]/6, 1295.2 [M + $5H^+$]/5, 1618.8 [M + $4H^+$]/4, 2158.0 [M + $3H^+$]/3; found 1079.7, 1295.1, 1618.8, 2158.8.

Succinimidyl BP-Pen (11). Bis(2-pyridylmethyl)pentanoic acid **3** (0.40 g, 1.3 mmol), EDC (1.04 g, 6.7 mmol), NHS (0.77 g, 6.7 mmol), and 5 mL of acetonitrile were combined in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the container was crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor at 100 °C for 10 min. The crude reaction mixture was concentrated using a rotary evaporator. The residue was dissolved in CH_2Cl_2, which was then extracted with water (50 mL × 2). The organic phase was concentrated to dryness leaving a viscous orange liquid. The residue was then dissolved in MeOH, filtered, and concentrated to dryness. The residue was dissolved in a minimum volume of CH_2Cl_2 and the desired product isolated by silica chromatography using a Biotage SP1 purification system and a solvent gradient of 3/97 (v/v) $MeOH/CH_2Cl_2$ to 20/80 $MeOH/CH_2Cl_2$ (v/v). The product (0.41 g, 77%) was an orange viscous liquid. R_f (CH_2Cl_2/MeOH, 90:10, v/v): 0.65. High resolution ES MS(+) calcd for $C_{21}H_{25}O_4N_4$ (M + H^+) m/z 397.1876, found 397.1872. ^{1}H NMR δ (500.13 MHz, $CDCl_3$): 8.45 (d, J = 5.5, 2H, PyH), 7.59 (t, J = 7.5, 2H, PyH), 7.45 (d, J = 7.5, 2H, PyH), 7.07 (t, J = 5.5, 2H, PyH), 3.74 (s, 4H, $PyCH_2$), 2.74 (s, 4H, succinimidyl CH_2), 2.50 (t, J = 6.0, 2H, CH_2N), 2.45 (t, J = 7.0, 2H, CH_2CO_2N), 1.68 (t, J = 7.0, 2H, CH_2), 1.59 (m, 2H, CH_2). ^{13}C NMR δ (125.76 MHz, $CDCl_3$): 169.1, 168.5, 159.8, 148.9, 136.4, 122.9, 121.9, 60.4, 53.2, 30.6, 26.1, 25.6, 22.3.

BP-Pen-AHx (12). Compound **11** (250 mg, 0.63 mmol) was combined with 6-aminocaproic acid (496 mg, 3.78 mmol) and 5 mL of acetonitrile in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the container was crimp-sealed. The mixture was heated in a Biotage Initiator 60 microwave reactor at 120 °C for 8 min. The precipitate (unreacted aminocaproic acid) was removed by filtration, and the filtrate was evaporated to give a pale-yellow residue. Purification by silica gel chromatography using a solvent gradient $MeOH/CH_2Cl_2$ 10:90 to 15:85 (v/v) as the eluent gave a pale-yellow oil (196 mg, 75%). R_f (CH_2Cl_2/MeOH, 90:10, v/v): 0.40. High resolution ES MS(+) calcd for $C_{23}H_{33}O_3N_4$ (M + H^+) m/z 413.2553, found 413.2571. ^{1}H NMR δ (500.13 MHz, $CDCl_3$): 8.48 (d, J = 5.0, 2H, PyH), 7.65 (dt, J = 1.5 and 8.0, 2H, PyH), 7.48 (d, J = 8.0, 2H, PyH), 7.16 (t, J = 6.0, 2H, PyH), 6.56 (m, 1H, NH), 3.80 (s, 4H, $PyCH_2$), 3.22 (q, J = 6.5, 2H, $NHCH_2$), 2.53 (t, J = 6.5, 2H, CH_2N), 2.30 (t, J = 7.0, 2H, CH_2CO_2H), 2.06 (t, J = 7.0, 2H, CH_2CONH), 1.61 (m, 2H, CH_2), 1.51 (m, 6H, CH_2), 1.37 (m, 2H, CH_2). ^{13}C NMR δ (125.76 MHz, $CDCl_3$): 176.8, 173.5, 158.9, 148.4, 137.2, 123.8, 122.5, 59.4, 53.6, 39.1, 36.2, 34.4, 29.0, 26.3, 25.3, 24.5, 23.5.

Succinimidyl BP-Pen-AHx (13). Compound **12** (100 mg, 0.24 mmol), EDC (230 mg, 1.2 mmol), and NHS (138 mg, 1.2 mmol) in 3 mL of acetonitrile were combined in a 2−5 mL Emery's process vial along with a magnetic stir bar, and the container was crimp-sealed. The sample was heated using a Biotage Initiator 60 microwave reactor at 120 °C for 10 min. The crude reaction mixture was concentrated using a rotary evaporator, dissolved in CH_2Cl_2, and extracted with water (25 mL × 2), and the organic phase was concentrated to give a dark-yellow oil. The residue was then dissolved in CH_2Cl_2 and purified by silica gel chromatography using 10/90 $MeOH/CH_2Cl_2$ (v/v) as the eluent. The product (110 mg, 89%) was isolated as a yellow oil. R_f(CH_2Cl_2/MeOH, 90:10, v/v): 0.50. High resolution ES MS(+) calcd for $C_{27}H_{36}O_5N_5$ (M + H^+) m/z 510.2716, found 510.2721. ^{1}H NMR δ (500.13 MHz, CD_3OD): 8.43 (d, J = 4.5, 2H, PyH), 7.79 (t, J = 7.5, 2H, PyH), 7.62 (d, J = 8.0, 2H, PyH), 7.27 (t, J = 6.0, 2H, PyH), 3.81 (s, 4H, $PyCH_2$), 3.15 (t, J = 6.7, 2H, CH_2NHCO), 2.82 (s, 4H, succinimidyl CH_2), 2.61 (t, J = 7.2, 2H, CH_2N), 2.56 (m, 2H, CH_2CO_2N), 2.12 (m, 2H, CH_2CONH), 1.73 (m, 2H, CH_2), 1.55 (m, CH_2), 1.44 (m, 2H, CH_2). ^{13}C NMR δ (125.76 MHz, CD_3OD): 175.9, 171.8, 170.2, 160.6, 149.4, 138.6, 124.9, 123.8, 61.1, 55.4, 40.0, 36.8, 31.5, 29.8, 27.6, 27.0, 26.5, 25.3, 24.8.

BP-Pen-AHx-DBI (9). Method A. A solution of **2** (83 mg, 13 μmol) and **11** (30 mg, 76 μmol) in DMSO (1.25 mL) containing DIPEA (25 μL) was stirred for 4 h at room temperature. The reaction mixture was then transferred to a centrifuge vial containing 15 mL of CH_3CN, and Et_2O was added slowly until a white precipitate formed. The precipitate was isolated by centrifugation at 3500 rpm for 30 min at 5 °C, and the resulting pellet was washed twice with 100% CH_3CN and reisolated by centrifugation. The pellet was then dissolved in 75/25 H_2O/CH_3CN containing 0.1% TFA, and the desired product was isolated by preparative reverse-phase HPLC. The fractions containing **9** were collected and concentrated by rotary evaporation (37 °C) to remove most of the CH_3CN. Following lyophilization, BP-Pen-AHx-DBI (53 mg, 64%) was obtained as a white powder. Analytical HPLC (Phenomenex Gemini C_{18} column 100 mm × 4.6 mm, method A) t_R = 9.03 min. MALDI TOF MS(+) calcd for $C_{290}H_{430}O_{83}N_{69}S_6$ (M + H^+) m/z 6403.3; found, 6403.1. ES MS(+) calcd 1285.2 [M + $4H^+$ + NH_4^+]/5, 1601.5 [M + $4H^+$]/4, 2135.0 [M + $3H^+$]/3; found, 1285.2, 1601.4, 2134.8.

Method B. Coupling with DBI. A solution of **1** (41 mg, 6.8 μmol) and **13** (35 mg, 68.7 μmol) in DMSO (0.4 mL) containing DIPEA (5%) was stirred for 4 h at room temperature. The reaction mixture was then transferred to a centrifuge vial containing 15 mL of CH_3CN. Then Et_2O was added slowly until a white precipitate formed. The precipitate was isolated by centrifugation at 3500 rpm for 30 min at 5 °C, then washed twice with 100% CH_3CN followed by centrifugation. The purification procedure was the same as in method A. Following lyophilization, BP-Pen-AHx-DBI (25 mg, 57%) was obtained as a white powder.

Digestion Studies. Digestion studies were performed using a previously reported procedure for derivatized insulin.[10] To 250 μL of compound **6** (2.5 mg/mL) in PBS was added 25 μL of 0.4 M aqueous NH_4HCO_3 in a plastic conical vial, and the mixture was agitated gently. To this was added 5 μL of 45 mM aqueous dithiothreitol, and the mixture was incubated for 15 min at 50 °C. After the mixture was cooled to room temperature, 5 μL of 100 mM aqueous iodoacetamide was added and the solution agitated periodically over 15 min. An aliquot (56 μL) was mixed with 28 μL of 0.4 M NH_4HCO_3 in 8 M aqueous urea and the analysis performed by LCMS (ES+).

The remaining solution from the dithiothreitol experiment was transferred to a glass vial. To this was added 2.5 μL of endoproteinase-Glu-C in water, and the mixture was incubated for 16 h at 37 °C. An aliquot (56 μL) was mixed with 28 μL of 0.4 M NH_4HCO_3 in 8 M aqueous urea and the analysis performed by LCMS (ES+).

In Vitro Biochemical Assays. Chinese hamster ovary cells stably transfected to express a large number of human insulin receptors (CHO-hIR cells) were prepared following the procedure of Ebina et al.[35] Human embryonic kidney (HEK) cell lines were obtained from ATCC (HEK-293) and were stably transfected to allow expression of a large number of human insulin receptors (hIR-293 cells) using the transfection method described by Marin-Hincapie and Garofalo.[36] H-4-IIE rat hepatoma cells were obtained from HTCC.

^{125}I-Insulin Competitive Binding Assay. Stock solutions of recombinant human insulin (Aventis, lot A0136-1) or Re-BP-AHx-insulin of varying concentrations were prepared in 10 mM HCl. An aliquot (25 μL) of either unlabeled recombinant human insulin (Aventis, lot A0136−-1) or Re-BP-AHx-insulin was added to 25 μL of ^{125}I-insulin (Amersham, 0.8 μCi/mL in 0.5% BSA in PBS) and 25 μL of 0.5% BSA in PBS. hIR-293 cells (100 μL, 1 $\times$ 10^6/mL) in 0.5% BSA in PBS was added and the solution incubated at room temperature for 60 min. The cells were isolated and washed three times with cold 0.5% BSA in PBS (3.5 mL per wash) and the cells counted on a γ counter. The concentration of the insulin for a 50% displacement was calculated using nonlinear regression analysis (Prism, version 4.0).

Insulin Receptor Autophosphorylation Assay. The CHO-hIR cells were serum deprived for 1 h, then incubated with either human insulin (Aventis) or Re-BP-AHx-insulin at various concentrations for 10 min. Cells were then lysed in a buffer consisting of 50 mM HEPES, pH 7.4, 150 mM NaCl, 1% NP-40, 0.1% deoxycholate, 0.1% SDS, 1 mM Na_3VO_4, 0.1 mM EGTA, and 1 Roche EDTA-free protease inhibitor cocktail tablet (per 50 mL volume). Lysates were clarified by centrifugation at 100000*g* for 5 min at 4 °C and applied to 96-well plates coated with anti-insulin receptor monoclonal antibodies (29B4 antibody). The extent of autophosphorylation was determined by quantitation of the binding of a second antibody directed against phosphotyrosine residues (HRP-conjugated PY20, Oncogene Research Products) using a coupled horseradish peroxidase reaction (enzyme-linked immunosorbent assay (ELISA)), and measurements were obtained by monitoring the colorimetric reaction using a UV spectrophotometer. Additional experimental details on the ELISA procedures can be found in the Supporting Information.

Akt1 Activation Assay. Confluent H4IIE cells were serum deprived for 4 h in serum containing β-mercaptoethanol and 0.25% bovine serum albumin. Triplicate wells were treated with human insulin (Aventis) or Re-BP-AHx-insulin at various concentrations for 10 min. Cells were lysed in 0.5 mL 1$\times$ cell lysis buffer (Cell Signaling, no. 7160) and cells broken open by brief sonication. Lysates were collected and centrifuged for 10 min at 14 000 rpm. Supernatants were recovered and diluted 1:1 with sample diluent, and phospho-Akt1 was quantitated in 100 μL of each diluted supernatant using the PathScan phospho-Akt1 (Ser473) ELISA (Cell Signaling, no. 7160) kit.

Radiochemistry. *Caution*: ^{99m}Tc is radioactive and should only be handled in a licensed facility using the appropriate shielding.

B^1-[^{99m}Tc(CO)$_3${bis(2-pyridylmethyl)pentanoyl-6-aminohexanoyl)}]insulin (^{99m}Tc-BP-Pen-AHx-insulin, 10). Boranocarbonate (8.5 mg), K^+/Na^+ tartrate (15 mg), $NaB_4O_7\cdot 10H_2O$ (3 mg), and Na_2CO_3 (4 mg) were added to 2−5 mL Emery's process vial along with a magnetic stir bar. The vial was crimp-sealed and purged with argon. Approximately 1 mL of $^{99m}TcO_4^-$ in saline from a ^{99}Mo/^{99m}Tc generator (1.11−1.85 GBq, 30−50 mCi) was added, and the sample was heated at 130 °C in a microwave reactor for 3 min. The solution containing $[^{99m}Tc(CO)_3(OH_2)_3]^+$ was cooled to room temperature, and 2.5 N HCl was carefully added to bring the pH of the solution to 6.5. Compound **9** (2 mg, 312 nmol) was dissolved in 30/60 (v/v) CH_3CN/H_2O (0.25 mL) and added by syringe to the solution of $[^{99m}Tc(CO)_3(OH_2)_3]^+$, and the mixture was heated in an aluminum heating block at 45 °C for 90 min. The crude reaction mixture was then transferred to a 20 mL scintillation vial and concentrated to dryness using a Biotage V10 evaporator. To the dried sample, 700 μL of TFA containing 5% (v/v) anisole was added, and the sample was dissolved using the redissolve mode on the V10 evaporator. After 10 min most of the TFA was removed using the evaporator and the sample dissolved in a solution of 25/75 (v/v) CH_3CN/H_2O containing 0.1% TFA, which was injected into an equilibrated semipreparative reversed-phase C18 column (Phenomenex Gemini 500 mm $\times$ 9.4 mm). The radioactive peak corresponding to the desired product was then collected into a 20 mL scintillation vial and concentrated to dryness using the V10 evaporator (T = 37 °C). The final purified product was dissolved in sterile phosphate buffered saline containing 1% bovine serum albumin (BSA) and filtered through a 0.2 μm filter (decay-corrected yield of 30 $\pm$ 11%, n = 4).

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Supporting Information Available: Additional experimental details on the ELISA for the IR and IR autophosphorylation procedures and characterization data (HPLC, MS, NMR) for novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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