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Preparation and Biological Properties of Biotinylated PhTX Derivatives

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Abstract—We report the synthesis of several highly functionalized biotinylated philanthotoxin (PhTX) analogues (**7**, **8**, **10**, **13**–**16**) designed on the basis of earlier structure–activity relationship studies. Despite the extensive modifications, the binding to nicotinic acetylcholine receptor (nAChR) is in the low micromolar range according to an inhibition assay using ^{3}H-thienylcyclohexyl-piperidine (TCP). A patch clamp functional assay gave comparable results. Compounds exemplified by **16**, which consists of a biotinylated ligand linked to a bifunctional photoaffinity probe (BPP), represent a new type of probe which should find use in photo-cross-linking studies of ligand–receptor interactions.

Introduction

Recognition of the gating mechanism of ion channels and interests in the three-dimensional structural features of these proteins have stimulated active studies in recent years.[1–3] Neurotoxins are powerful probes in these studies,[4] and in many cases chemically modified neurotoxins are providing considerable structural information of the corresponding receptors.[5,6] Philanthotoxin-433 (**1**) (PhTX-433,[7,8] the numerals denote number of methylene groups between the amino groups, Fig. 1), a polyamine amide toxin isolated from the venom of *Philanthus triangulum*, and its analogues are non-competitive antagonists of the nicotinic acetylcholine and glutamate receptors (nAChR, GluR).[9–14] Analogues carrying fluorine and porphyrin labels have also been synthesized and submitted to biophysical and spectroscopic studies in order to investigate their interactions with nAChR,[15] while PhTX derivatives with ^{125}I labels have been employed for preliminary photoaffinity labeling studies of nAChR.[16]

We recently developed a new bifunctional photoaffinity probe (BPP),[17] which should find use in photo-cross-linking studies of ligand/receptor interactions. The bifunctional photoaffinity probe BPP (Fig. 2), with two different photolabile groups at sites A and B, was designed to serve as a new general probe for cross-linking studies of ligand/receptor complexes in the following manner:[17] (1) The ligand, which is attached to a biotin group via a spacer, and the receptor are incubated to form the complex. (2) Irradiation at 350 nm under neutral conditions leads to carbene generation at site A which cross-links to the protein receptor. (3) The receptor is cleaved chemically or enzymatically. (4) The cross-linked and non-cross-linked peptide fragments are separated using the biotin tag by avidin affinity chromatography. (5) The separated cross-linked peptide fragment(s) is irradiated again at 350 nm, but under mildly basic conditions, upon which the BPP group is cleaved at site B, thus leaving only the nitrophenolic marker cross-linked to the peptide(s), which is then sequenced by MS.[18,19] We have also shown that BPP can be linked to a primary amino group by reductive amination of the Schiff base formed between BPP aldehyde 2-[5-nitro-3-(3-trifluoromethyl-1,2-diazirin-3-yl)-phenoxy]ethanal **19** (Scheme 1) and the primary amine. Earlier studies showed that introduction of terminal alkyl groups in the polyamine chain did not decrease the binding activity so far as the amino groups are not transformed into amides.[9,15,17] Therefore, it can be expected that a BPP-linked molecule such as that depicted in Figure 2 or Scheme 1, **16**, despite multiple modifications, should retain reasonable activity. Assay results indeed confirmed this speculation (see below). This unique molecule could play an important role in determining the cross-linking sites in ligand/receptor interaction studies.

Key words: Philanthotoxin; biotin-labeling; nAChR; photoaffinity.
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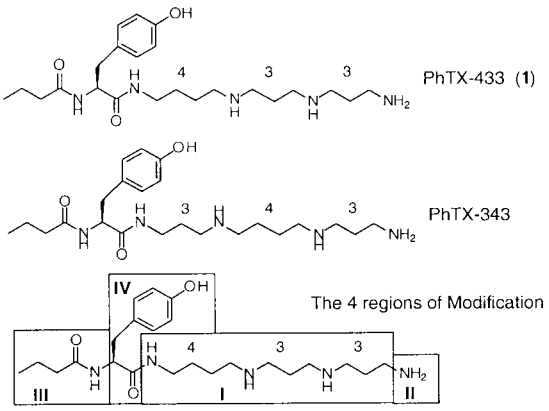


Figure 1. Structures of the natural philan-thotoxin-433 (PhTX-433) and PhTX-343.

Extensive studies on structure–activity relationship (SAR) of PhTX with respect to nAChR and GluR have been carried out systematically by dividing PhTX into four regions I–IV.[9,13,15,20,21] Since the activity of PhTX-343 and PhTX-433 are comparable,[7] the symmetric spermine was incorporated as the polyamine chain for ease of synthesis (region I), and then elongated with a lysine unit (region II) to enhance binding.[9] Replacement of the butyryl amide moiety (region III) with longer aliphatic amides enhances binding activity while substitution of this moiety with polar functions results in loss of activity.[22] On the other hand, an excessively long hydrophobic chain led to poor aqueous solubility, thus making such analogues unsuited for biological applications.

Here we report the preparation and binding properties of bioactive biotinylated philanthotoxins, including analogue **16** which incorporates the BPP group. Biotin, with an affinity to avidin of $K_d = 10^{-15}$, has been used extensively in bioorganic studies[23–25] and subsequent cross-linking studies to elucidate ligand–receptor 3-D interactions. This type of biotinylated PhTXs and other ligands can be utilized in a variety of experiments, including purification of receptors or selective sequestering of the photo-cross-linked peptide fragments by agarose-avidin affinity chromatography. In order to incorporate a biotin moiety for affinity chromatography purposes and yet cope with the solubility problem, a polar triethylene glycol spacer was inserted between the biotin moiety and the long hydrophobic acyclic chain (region III). By this means, compound **13**, which was expected to be the least soluble, was soluble in water at concentrations around 1 mM.

The length of the spacer should be sufficiently long so that both the biotin and PhTX moieties have sufficient space to reach their respective binding pockets.[26] The biotin–avidin complexes are strongly bound ($K_d = 10^{-15}$) and the dissociation process is slow. In cases where the BPP group is used for cross-linking studies, it is not necessary to dissociate the biotin-linked ligand–peptide fragment from the avidin column since the peptide is cleaved at site B of BPP, so that the cross-linked peptide fragment(s) linked to the nitrophenol marker is eluted. However, in cases where BPP is not employed, the biotin-ligand-peptide fragment has to be dissociated from the avidin column. In order to cope with such cases, analogues with a weaker biotin/avidin association were prepared by converting the biotin into their sulfoxides based on the work of Leonard.[27] The following analogues have been synthesized: bio-C_{10}-PhTX343 (**7**), its lysine adduct bio-C_{10}-PhTX343-Lys (**8**), the diastereomeric pair of sulfoxides (O)-bio-C_{10}-PhTX343 (**10**α and **10**β), the iodine derivative bio-C_{10}-PhTX(I_2)343 (**13**), the lysine adduct bio-C_{10}-PhTX(I_2)343-Lys (**14**), its β-sulfoxide β(O)-bio-C_{10}-PhTX(I_2)343 (**15**), and finally bio-C_{10}-PhTX343-BPP (**16**).

Results and Discussion

The syntheses are summarized in Schemes 1 and 2. Alcohol **2** was prepared from triethylene glycol by: (i) mono-acetylation of triethylene glycol (Ac_2O, Py, CH_2Cl_2, 74%); (ii) mesylation of the resulting alcohol (MsCl, Et_3N, CH_2Cl_2, 73%); (iii) substitution by azide

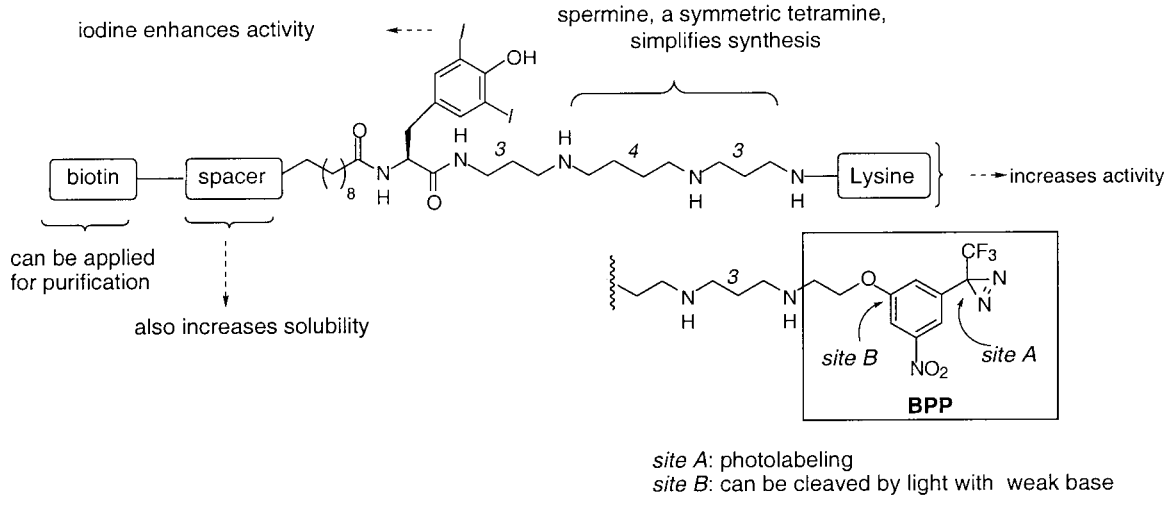
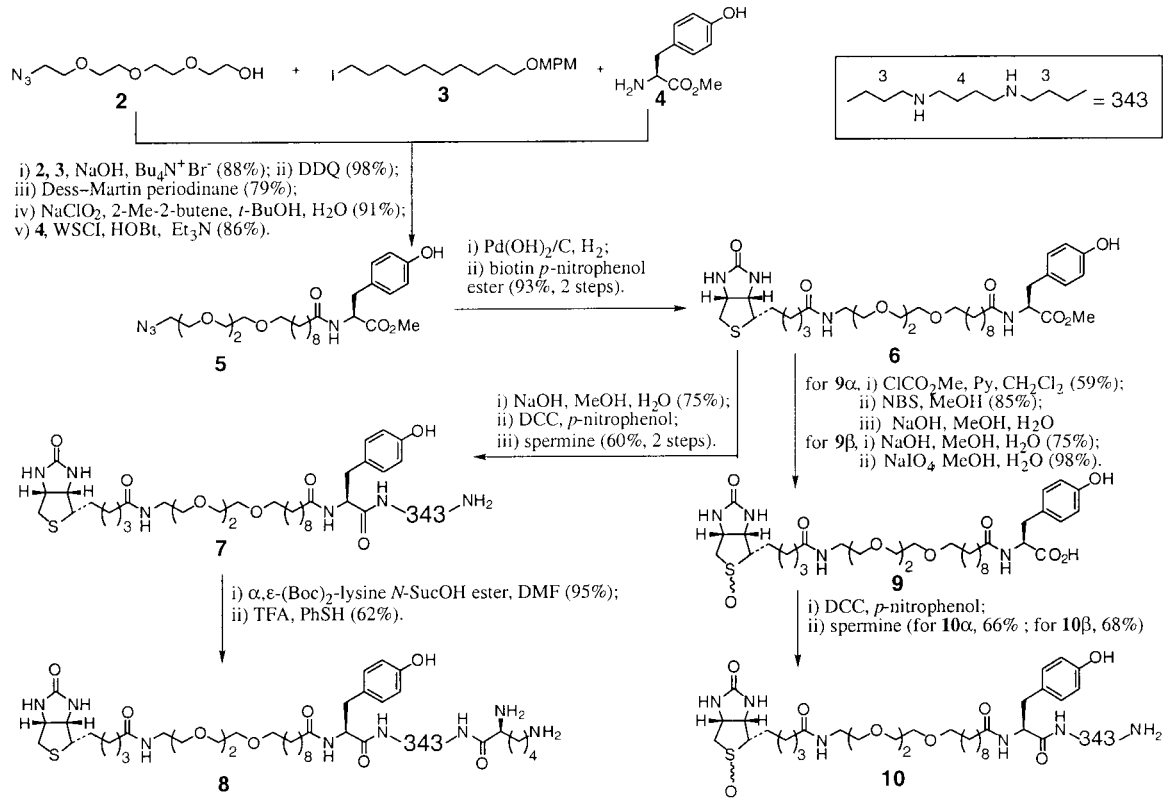
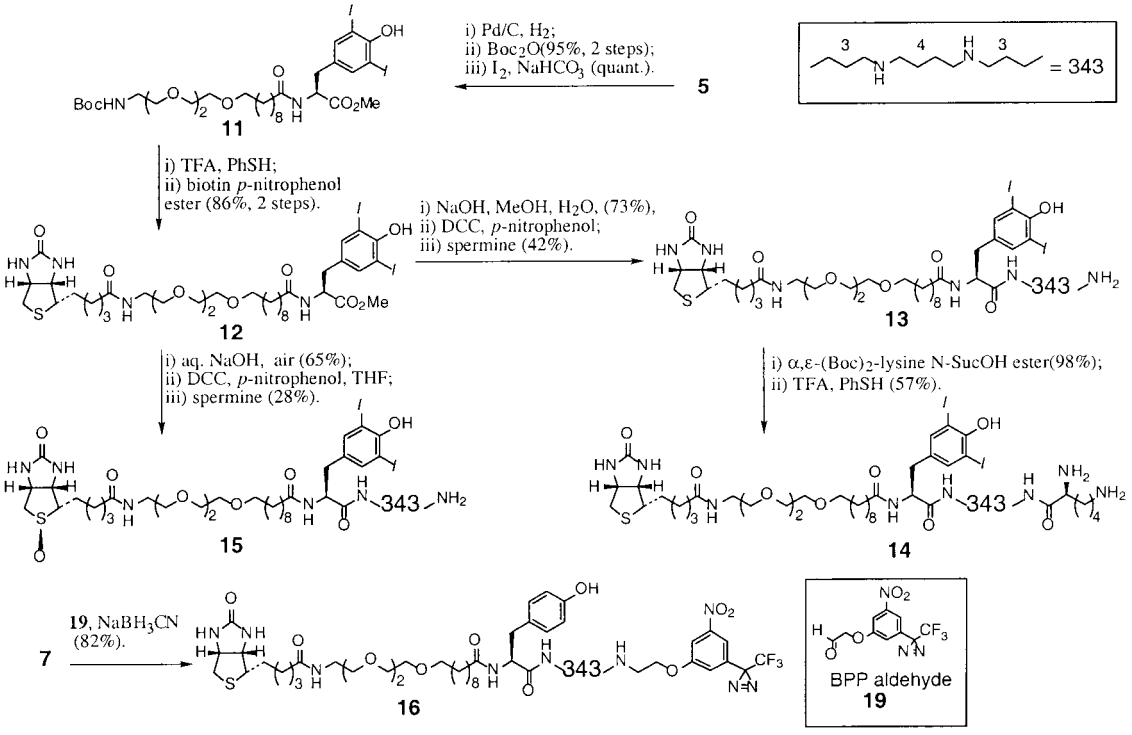


Figure 2. Design of Philanthotoxin derivatives.



Scheme 1.



Scheme 2.

(NaN_3, H_2O, EtOH); and (iv) deacetylation (NH_4OH, MeOH, 97%, 2 steps). A straightforward manipulation of 1,10-decanediol provided **3** via: (i) mono-MPM ether formation (NaH, MPMCl, DMF, 55%); (ii) mesylation of the resulting alcohol (MsCl, Et_3N, CH_2Cl_2, 99%); and (iii) substitution with iodine (NaI, acetone, 99%). After coupling of units **2** and **3** with aqueous NaOH in the presence of Bu_4NBr,[28] the MPM group was removed with DDQ.[29] The regenerated hydroxyl group was then transformed into a carboxylic group stepwise by Dess–Martin reagent[30] followed by $NaClO_2$. The carboxylic acid thus obtained was coupled with tyrosine methyl ester **4** employing combined usage of WSCI[31] and HOBt,[32] to give adduct **5**. Reduction of the azide group in **5** was achieved using palladium catalyzed hydrogenation, to provide the corresponding amine,[33] which was treated with biotin *p*-nitrophenyl ester[34] without chromatographic purification to yield bio-C_{10}-Tyr-OMe **6**. Spermine was then coupled by DCC after saponification of the methyl ester in **6**, to furnish bio-C_{10}-PhTx343 (**7**). Treatment of **7** with one equivalent of *N*α,-*N*ε-di-t-Boc-*L*-lysine hydroxysuccinimide ester gave rise to the coupling product in good yield. Removal of the Boc groups by TFA in the presence of thiophenol yielded bio-C_{10}-PhTX343-Lys (**8**).

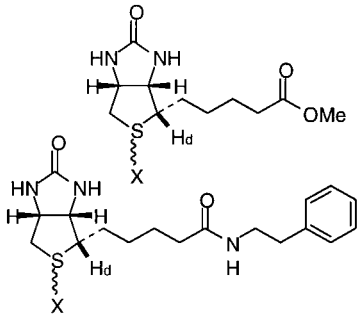
Compound **6** was also transformed into the diastereomeric sulfoxides **9**α and **9**β. Stereoselective oxidation into α-sulfoxide **9**α was performed by the procedure of Leonard,[27] who reported that *N*-bromosuccinimide (NBS) oxidation of certain biotins in anhydrous methanol resulted in stereoselective formation of α-sulfoxybiotins. As it was found that simultaneous bromination of the *ortho* position of phenol moiety of tyrosine took place when **6** was treated with NBS, it was necessary to reduce the electron density of the aromatic ring. Thus the phenol function was first acylated as a methyl carbonate by $ClCO_2Me$ and pyridine in CH_2Cl_2, and the product submitted to NBS oxidation to yield the α-sulfoxide selectively in 85% yield; subsequent cleavage of the methyl carbonate and methyl ester under basic aqueous conditions provided the desired **9**α. The ^1H NMR spectra of the product showed that only a small amount of β-sulfoxide was formed (see below for assignment of sulfoxide stereochemistry). β-Sulfoxide **9**β was synthesized by saponification of **6** under aqueous basic conditions, followed by $NaIO_4$ treatment which occurred stereoselectively from the convex side of the biotin bicyclic ring system, giving rise to β-selective oxidation. Linking of spermine to **9**α and **9**β was performed in the same manner as in the synthesis of **7**, giving rise to the diastereomeric pair of α(O)-bio-C_{10}-PhTX343 (**10**α and its isomer **10**β, respectively).

Synthesis of derivatives with iodine on the tyrosine rings was next carried out. Oxidation of the phenol group was performed before introducing the biotin-containing portion, since the sulfide function in biotin is more susceptible to most oxidative conditions than phenols. The azide group of **5** was therefore first transformed into the corresponding *N*-Boc protected amine by sequential palladium catalyzed reduction and protection by $(Boc)_2O$. It was found that treatment of the product with I_2 in the presence of $NaHCO_3$ resulted in formation of 2,6-diiodophenol, giving **11** in high yield. Deprotection of the Boc group followed by coupling with biotin *p*-nitrophenol ester gave rise to **12**. After saponification of the methyl ester moiety of **12** with aqueous NaOH, coupling with spermine and then with lysine proceeded smoothly, producing Bio-C_{10}-PhTX(I_2)343 (**13**) and Bio-C_{10}-PhTX(I_2)-343-Lys (**14**). It is worth noting that when hydrolysis of the methyl ester in **12** was performed under ambient atmosphere, β-stereoselective oxidation of the biotin part took place to give the β-sulfoxide, which was then reacted with spermine to give β(O)-bio-C_{10}-PhTX(I_2)343 (**15**). Finally, treatment of **7** with BPP aldehyde and sodium cyanoborohydride in MeOH in the presence of TFA gave the desired product **16** in 82% yield.

The stereochemistry of biotin sulfoxide moieties was assigned as follows (Table 1). Leonard demonstrated the α-selective sulfoxide formation of the biotin function employing NBS in anhydrous MeOH.[27] In order to ascertain the stereochemistry in **10**α and **10**β biotin methyl ester (**17**) and biotin β-phenethyl amide (**18**) were oxidized with NBS and with $NaIO_4$. Both oxidations proceeded stereoselectively to yield products with similar but not identical ^1H NMR spectra; the spectra of **10**α and **10**β were also close but not identical. The configurations were determined by comparison of chemical shifts as well as coupling constants in the ^1H NMR spectra.[35,36] The Hd signal of the NBS oxidation product **17**α appeared at a higher field compared to **17**, while all other signals of the biotin moiety underwent a low field shift in both **17**α and **17**β. The same was true for **18** and **10** series. In addition, clear differences in coupling constants were also observed for $J_{Ha–Hc}$. Namely, those of **17**, **18**, and **7** were in the range from 0.4 to 0.8 Hz, while those of the sulfoxides prepared by NBS (α-sulfoxide) and $NaIO_4$ (β-sulfoxide) were 0.0 and 2.2–2.5 Hz, respectively. As there was no exception in the relationships between oxidation methods and ^1H NMR data, sulfoxides obtained by NBS and $NaIO_4$ oxidation were assigned α- and β-configurations, respectively.

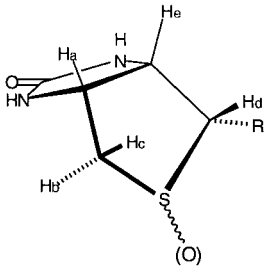
Biotin functions were introduced into the molecules in order to take advantage of the biotin–avidin affinity in chromatographic separation. Although it was expected that the terminal biotin group in these compounds is sufficiently separated from the bulk of the molecule so that it can bind with avidin, it was necessary to check this point because it was possible that the strongly basic polyamine moiety could interact with avidin to interfere with the binding. Thus, a displacement assay using the dye 2-(4′-hydroxyphenylazo)-benzoic acid (HABA)[37] was employed to measure their affinity to avidin. It is known that HABA also associates with avidin ($K_a = 6\times10^{-6}$ M at pH 4.7). Upon complexation with avidin, HABA exhibits intense UV absorption with $\varepsilon = 35{,}500$ at 500 nm, while the released free HABA shows $\varepsilon = 480$ at 500 nm. Thus when compounds with stronger affinity for avidin than HABA are added to the avidin–HABA complex, HABA is released from avidin, giving rise to a change in the UV profile. Titration of the avidin–HABA complex with aqueous solutions of

Table 1. ^{1}H NMR Data of some biotin derivatives and their sulfoxides



17: X = lone pair electrons
17α: X =α-O
17β: X =β-O

18: X = lone pair electrons
18α: X =α-O
18β: X =β-O



	Methods (yield %, α:β)	Ha[a]	Hb	Hc	Hd	He	J_{Ha-Hc} (Hz)
17	Sulfide	4.51	2.94	2.75	3.22	4.32	0.8
17α	A[b] (90%, > 10:1)[d]	4.91	3.01	3.40	2.87	4.76	0.0
17β	B[c] (85%,1:4)	4.70	3.09	3.55	3.17	4.61	2.2
18	Sulfide	4.51	2.95	2.63	3.20	4.30	0.8
18α	A[b] (82%, > 10:1)[d]	4.87	3.00	3.41	2.83	4.72	0.0
18β	B[c] (92%,1:10)	4.80	3.21	3.63	3.23	4.64	2.4
7	Sulfide	4.51	2.94	2.72	3.24	4.32	0.4
10α	A[b] (92%,16:1)	4.86	3.02	3.42	2.83	4.73	0.0
10β	B[c] (66%, < 1:10)[d]	4.70	3.09	3.54	3.21	4.61	2.5

Chemical shifts and coupling constants are reported in ppm (versus TMS) and Hz, respectively (400 MHz in CD_3OD). Small differences in chemical shifts (max ± 0.10 ppm) from reported data in authentic methyl ester series were observed. Our own observations are reported here.
[a]For convenience, the numbering for biotin is arbitrary and does not follow the IUPAC nomenclature.
[b]Condition (A) NBS in anhydrous MeOH, room temperature.
[c]Condition (B) $NaIO_4$ in aqueous MeOH, room temperature.
[d]No isomer was observed by ^{1}H NMR. The selectivities were determined right after oxidations by NMR spectra.

biotinylated PhTXs resulted in a linear decrease in A_{500} against the volume of PhTX solution added. This showed that all compounds (**7**, **8**, **10**, **13**–**16**) formed stable avidin complexes with dissociation constants below the sensitivity limit of this method ($K_d < 10^{-9}$),[38] and could be used in avidin affinity chromatography to facilitate photoaffinity labeling studies. However, the affinity was not high enough for purposes of receptor purification.[39]

A radioligand competition assay was next performed to determine the binding activity of analogues with nAChR based on the protocol developed by Eldefrawi and co-workers with minor modifications.[15,40] In this study, the commercially available ^{3}H-thienylcyclohexylpiperidine (^{3}H-TCP), a recently characterized potent noncompetitive antagonist (channel blocker) of nAChR,[41] was employed as the radioligand instead of ^{3}H-histrionicotoxin, which is difficult to obtain. The sensitivity and reproducibility of the system were confirmed using known antagonists of nAChR including amantadine, chlorpromazine and trifluoperazine.[15,40] IC_{50} values of all compounds tested were found from the inhibition curves obtained (Fig. 3) and are shown in Table 2. All experiments were carried out in triplicate with < 10% relative errors.

The results of these assays can be summarized as follows.

(1) All biotinylated analogues of PhTX exhibit stronger binding than native PhTX-433, with IC_{50} values in the low μM range. (2) Compound **6** without the PhTX moiety can be regarded as a control for the biotin-spacer chain. It is inactive. (3) However, linking of the biotin-spacer chain and the PhTX moiety as in **7** bio-C_{10}-PhTX343 gave rise to a 37-fold potentiation over PhTX 343. In contrast, the activity of the moiety lacking the biotin-spacer chain (i.e., C_{10}-PhTX343) was only 10-fold that of PhTX 343.[21] The reason for this synergistic enhancement is unclear. (4) Other biotinylated analogues **8**, **13**, **14**, **15**, and **16** also exhibit a 30- to 50-fold enhancement in activity over PhTX. (5) Results with sulfoxides are varied. In the case of **10α** and **10β** sulfoxidation significantly diminishes activity, whereas with **15** the activity remains more or less the same.

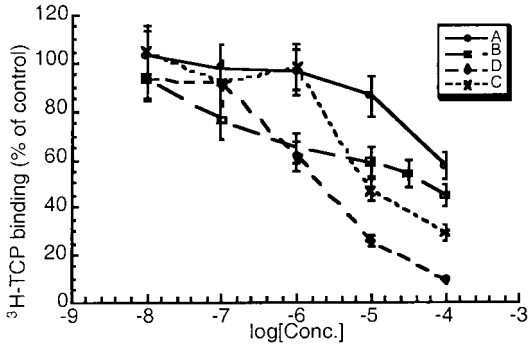


Figure 3. Selected inhibitory curves of philanthotoxin derivatives. (Conc.: concentration (M); A: **6**; B: PhTX-343; C: **7**·avidin complex; D: **7**.)

Table 2. Summary of binding activity of biotinylated PhTX derivatives with nAChR

	Compound	Free compounds		Avidin complexes	
		IC_{50} (μM)	Relative[a] activity	IC_{50} (μM)	Relative activity
	PhTX-433	75	1	–	1
	PhTX-343	75	1	–	1
6	bio-C_{10}-Tyr-OMe	> 300	< 0.25	> 300	< 0.25
7	bio-C_{10}-PhTX343	2.0	37	6.0	12.5
8	bio-C_{10}-PhTX343-Lys	1.5	50	35	2.2
10α	α(O)-bio-C_{10}-PhTX343	8.0	9.4	18	4.2
10β	β(O)-bio-C_{10}-PhTX343	14	5.3	33	2.3
13	bio-C_{10}-PhTX(I_2)343	2.6	29	15	5.0
14	bio-C_{10}-PhTX(I_2)343-Lys	1.5	50	29	2.6
15	β(O)-bio-C_{10}-PhTX(I_2)343	1.4	53	20	3.7
16	bio-C_{10}-PhTX343-BPP	2.5	30	22	3.5

[a]Relative activities were calculated as the ratio: (IC_{50} of PhTX433):(IC_{50} of new compounds). The lower the IC_{50}, the higher the relative activity.

(6) SAR of PhTX showed that attachment of Lys in region 2 led to a sevenfold enhancement in activity,[21] whereas in the case of biotinylated derivatives (**7** versus **8**, **13** versus **14**), the enhancement is less than twofold. Bis-iodination of the tyrosine moiety increased the activity by eightfold in PhTX;[42] however, in the biotinylated derivatives, the activity remains unchanged (**8** versus **14**) or is slightly decreased (**7** versus **13**) after iodination. (7) Note that the activity of biotinylated analogue **16** carrying the BPP moiety is 30-fold that of PhTX. (8) Relative activities of the avidin complex of biotinylated PhTX analogues are weaker than the non-complexed free forms but are still noteworthy. Preliminary patch clamp assays performed by Dr. Matthew Brierley, Nottingham University, on TE671 cells with overexpressed nAChR receptor gave the following results: at a concentration of 10^{-6} M acetylcholine and 100 mV, the inhibition on the transmembrane currents caused by 10 μM solutions of PhTX analogues was: **1** 47.4%, **6** 17.0%, **7** 85.4%, **10α** 62.0%, **10β** 52.1%, and **14** 58.6%. The results from this functional assay are in good agreement with binding assay results.

In summary, the synthesis of a series of novel biotinylated PhTX analogues has been carried out. Binding assays showed that these compounds and their avidin complexes exhibit reasonable binding activities against nAChR. The 30-fold enhanced activity of analogue **16** incorporating the bifunctional photoaffinity probe BPP should be noted; this demonstrates that such analogues should be useful for streamlining the frequently tedious photo-cross-linking studies of ligand–receptor 3-D structural studies.

Experimental

General

Melting points are uncorrected. ^{1}H NMR spectra were measured on Bruker DMX 300, DMX 400, or DMX 500 spectrometers. Chemical shifts are reported in ppm down fields from the peak of tetramethylsilane as an internal standard. Splitting patterns are designated as "s, d, t, q, quint, m, and br", indicating "singlet, doublet, triplet, quartet, quintet, multiplet and broad", respectively. IR spectra were recorded on Perkin–Elmer Paragon 1000FT IR spectrophotometer with NaCl cell or KBr pellets. Optical rotations were measured by Jasco DIP-1000 digital polarimeter. Measurements of high and low resolution mass (MS) spectra were performed on Nermag R10-10 or Jeol JMS-DX 303HF mass spectrometers. FAB mass spectra were collected using 3-nitrobenzoylalcohol (NBA) as matrix. Analytical thin layer chromatography was carried out by precoated silica gel plates (Merck Art 7754). Prep TLC plates used were Analtech Uniplate silica gel GF Preparative Layers 20×20 cm (1000 microns). Silica gel used for column chromatography was Select Scientific silica gel Art 162824.

All reactions were carried out under rt and argon atmosphere unless specified. Throughout the experimental section, the following abbreviations are used: (Ac_2O) acetic anhydride; (MsCl) methanesulfonylchloride; (HOBT) 1-hydroxybenzotriazole; (WSCI) 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride; (DCC) dicyclo-hexylcarbodiimide; (Boc_2O) di-*t*-butyl dicarbonate; (PhSH) thiophenol; (THF) tetrahydrofuran; (*i*-PrOH) 2-propanol; (*i*-$PrNH_2$) isopropylamine; aqueous (aq). THF was distilled from sodium benzophenone ketyl before use. Commercially available anhydrous grade CH_2Cl_2 and DMF were purchased from Aldrich. Other solvents were used without further purification. α,ε-bis(*t*-butoxycarbonyl)lysine hydroxysuccinimide ester were purchased from Sigma.

2-(2-Azidoethoxy)ethoxyethanol (2). To a solution of triethylene glycol (6.00 mL, 6.78 g, 45.0 mmol) and pyridine (6.0 mL. 5.88 g, 74 mmol) in CH_2Cl_2 (30 mL) was added Ac_2O (3.00 mL, 3.24 g, 31.8 mmol) at 0 °C. The mixture was allowed to warm to rt and stirred for another 12 h. After concentration, the residue was purified by column chromatography (silica gel, acetone: CH_2Cl_2, 25:75) to afford 2-[2-(2-hydroxyethoxy)ethoxy]-ethyl acetate (4.40 g, 22.9 mmol, 74% based on Ac_2O) as an oil. IR (film) 3030, 2930, 1600, 1455, 1030, 905 cm^{-1}. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.10 (3H, s), 2.22 (1H, br), 3.63 (2H, dd, J = 4.0, 5.9 Hz), 3.70 (8H, m), 4.25 (2H, dd, J = 4.6, 5.9 Hz). MS (EI) m/z (%) 193 (9.3, MH^+), 87 (100, $AcOCH_2CH_2^+$). HRMS (EI) found 193.1074, calcd for $C_8H_{17}O_5$ (MH^+) 193.1076.

To a mixture of the above obtained acetate (1.92 g, 9.99 mmol) and Et_3N (1.8 mL, 13.9 mmol) in CH_2Cl_2 (10 mL) was added MsCl (950 μL, 1.38 g, 12.0 mmol) at −78 °C. After stirring at −78 °C for 2 h, the mixture was poured into water and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4, and concentrated in vacuo. The residue was purified with column chromatography (silica gel, acetone:CH_2Cl_2, 5:95) to give 2-[2-(2-methanesulfoxyethoxy)ethoxy]ethyl acetate (2.61 g, 9.67 mmol, 97%) as an oil. IR (film) 2880, 1740, 1250, 1175, 1055, 1030, 970 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 2.10 (3H, s), 3.09 (3H, s), 3.69 (6H, m), 3.79 (2H, dd, J = 3.9, 4.6 Hz), 4.23 (2H, dd, J = 3.9, 4.9 Hz), 4.40 (2H, dd, J = 3.6, 4.9 Hz). MS (EI) m/z (%) 271 (10, MH^+), 210 (3.7, [M^+-AcOH]), 166 (20, [M^+-$AcOCH_2CH_2OH$]), 123 (100, $MsOCH_2CH_2^+$). HRMS (EI) found 271.0850, calcd for $C_9H_{19}SO_7$ (MH^+) 271.0852.

The mesylate obtained above (4.00 g, 14.8 mmol) and sodium azide (2.30 g, 35.0 mmol) in a mixture of EtOH (35 mL) and H_2O (17 mL) was stirred at 80 °C for 3 h. After ethanol was removed in vacuo, the mixture was poured into AcOEt and washed with water. The organic layer was washed with brine, dried with Na_2SO_4, and concentrated. The residue was diluted with MeOH (20 mL) and 30% aq NH_4OH (20 mL), and the mixture was stirred for another 12 h. After concentration, the residue obtained was purified by column chromatography (silica gel, acetone:CH_2Cl_2, 25:75) to give **2** (2.52 g, 14.4 mmol, 97%, 2 steps) as an oil. IR (film) 3450, 2870, 2110, 1120 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$), δ 3.42 (2H, t, J = 5.2 Hz), 3.64 (2H, dd, J = 3.9, 5.9 Hz), 3.69 (6H, m), 3.76 (2H, dd, J = 3.9, 5.9 Hz), MS (EI) m/z (%) 176 (17, MH^+), 119 (100, [M^+-N_3CH_2]). HRMS (EI) found 176.1031, calcd for $C_6H_{14}N_3$ (MH^+) 176.1036.

10-Iododecanyl MPM ether (3). To a suspension of NaH (washed with hexane, 730 mg, 30.4 mmol) in DMF (20 mL) was added 1,10-decanediol (5.00 g, 28.7 mmol). After the mixture was stirred at 30 °C for 1 h, *p*-methoxybenzylchloride (MPMCl) (4.3 mL, 4.95 g, 31.6 mmol) was added and the whole mixture was stirred for another 6 h. The mixture was poured into water and extracted with ether. The ethereal solution was washed with brine, dried over Na_2SO_4, and concentrated. The residue obtained was purified with column chromatography (silica gel, AcOEt:hexane, 20:80) to give the 10-hydroxydecanyl MPM ether (4.61 g, 15.6 mmol, 55%) as a pellet. Mp 46.5–47.5 °C (from ether/hexane). IR (KBr), 3420, 2930, 2850, 1515, 1460, 1245, 1105, 1025 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (12H, m), 1.45 (1H, br), 1.62 (4H, m), 3.45 (2H, t, J = 6.7 Hz), 3.65 (2H, t, J = 6.7 Hz), 3.82 (3H, s), 4.45 (2H, s), 6.90 (2H, brd, J = 8.0 Hz), 7.29 (2H. brd, J = 8.0 Hz). MS (EI) m/z (%) 294 (12, M^+), 137 (30, $MeOC_6H_4CH_2O^+$), 121 (100, $MeOC_6H_4CH_2^+$). HRMS (EI) found 294.2199, calcd for $C_{18}H_{30}O_3$ (M^+) 294.2196.

A mixture of the mono MPM ether thus obtained (4.0 g, 13.6 mmol) and Et_3N (4.0 mL, 3.12 g, 30.9 mmol) in CH_2Cl_2 (20 mL) was stirred with MsCl (1.6 mL, 2.36 g, 20.7 mmol) at −78 °C. After 10 min, the cooling bath was removed and the mixture was further stirred for 1 h at rt. The mixture was poured into water and was extracted with ether. The ethereal solution was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified with column chromatography (silica gel, AcOEt:hexane, 20:80) to give 10-methanesulfoxydecanyl MPM ether (5.00 g, 13.4 mmol, 99%) as needles. Mp < 30 °C (from cold ether/hexane). IR (film, compound melted under measurement condition) 2930, 2850, 1515, 1360, 1250, 1170, 1100, 1030, 995 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (12H, m), 1.60 (2H, quint, J = 6.7 Hz), 1.76 (2H, quint, J = 6.7 Hz), 3.02 (3H, s), 3.45 (2H, t, J = 6.7 Hz), 3.83 (3H, s), 4.24 (2H, t, J = 6.7 Hz), 4.45 (2H, s), 6.90 (2H, brd, J = 8.0 Hz), 7.28 (2H, brd, J = 8.0 Hz). MS (EI) m/z (%) 372 (5.0, M^+), 137 (100, $MPMO^+$), 121 (100, MPM^+). HRMS (EI) found 372.1973, calcd for $C_{18}H_{32}O_5S$ (M^+) 372.1972.

A suspension of the above mesylate (5.00 g, 13.4 mmol) and NaI (12.0 g, 56 mmol, 4.2 equiv) in acetone (60 mL) was refluxed with stirring for 3 h. After removing the solvent, the residue was diluted with water containing small amount of $Na_2S_2O_3$ and extracted with ether. The ethereal solution was washed with brine, dried over Na_2SO_4, and concentrated. The residue was purified with column chromatography (silica gel, AcOEt:hexane, 4:96) to give **3** (5.35 g, 13.2 mmol, 99%) as an oil. IR (film) 2930, 2850, 1515, 1360, 1250, 1170, 1100, 1030, 995 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.35 (12H, m), 1.65 (2H, quint, J = 6.7 Hz), 1.84 (2H, quint, J = 6.7 Hz), 3.21 (2H, t, J = 6.7 Hz), 3.45 (2H, t, J = 6.7 Hz), 3.83 (3H, s), 4.45 (2H, s), 6.90 (2H, brd, J = 8.0 Hz), 7.28 (2H, brd, J = 8.0 Hz). MS (EI) m/z (%) 404 (27, M^+), 121 (100, $MeOC_6H_4CH_2^+$). HRMS (EI) found 404.1209, calcd for $C_{18}H_{29}O_2I$ (M^+) 404.1209.

***N*-10-(2-[2-(2-Aminoethoxy)ethoxy]ethoxy)decanoyl-(L)-tyrosine methyl ester (5).** A suspension of **2** (1.20 g, 6.25 mmol), **3** (3.50 g, 8.66 mmol), and tetrabutylammonium bromide (160 mg, 1.55 mmol) in 50% aq NaOH (10 mL) was stirred at 60 °C for 5 h. The mixture was poured into water and extracted with ether. The ethereal extract was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography (silica gel, AcOEt:hexane, 20:80) to give 10-(2-[2-(2-azidoethoxy)ethoxy]ethoxy)-decanyl MPM ether (2.47 g, 5.47 mmol, 88% based on **2**) as an oil. IR (film) 2930, 2860, 1510, 1250, 1100 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.29 (12H, m), 1.59 (4H, m), 3.41 (2H, t, J = 4.8 Hz), 3.45 (2H, t, J = 6.6 Hz), 3.46 (2H, t, J = 6.6 Hz), 3.60 (2H, m), 3.68 (8H, m), 3.82 (3H, s), 4,45 (2H, m), 6.90 (2H, brt, J = 8.7 Hz), 7.27 (2H, brt, J = 8.7 Hz). MS (CI, NH_3) m/z 469 ($M + NH_4^+$). HRMS (FAB) found 474.2944, calcd for $C_{24}H_{41}N_3O_5Na$ ($M + Na^+$) 474.2946.

A mixture of the above adduct (2.30 g, 5.10 mmol) and 2,3-dichloro-5,6-dicyano-1,4-benzoquinone (DDQ) (1.50 g, 6.60 mmol) in a mixture of CH_2Cl_2 (30 mL) and H_2O (3.0 mL) was stirred for 1 h. The mixture was then poured into water and extracted with ether. The ethereal

solution was washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified with column chromatography (silica gel, acetone:CH_2Cl_2, 10:90) to give 10-(2-[2-(2-azidoethoxy)ethoxy]ethoxy)decanol (1.66 g, 5.02 mmol, 98%) as an oil. IR (film) 3450, 2930, 2105, 1300, 1120 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.29 (12H, m), 1.56 (4H, m), 3.40 (2H, brt, $J=4.8$ Hz), 3.46 (2H, t, $J=6.9$ Hz), 3.60 (2H, m), 3.67 (10H, m). MS (EI) m/z (%) 332 (16, MH^+), 160 (base peak). HRMS (EI) found 332.2546, calcd for $C_{16}H_{34}N_3O_4$ (MH^+) 332.2551.

A solution of the obtained alcohol (1.66 g, 5.02 mmol) in CH_2Cl_2 (30 mL) was stirred with Dess–Martin reagent[30] (2.70 g, 6.53 mmol) for 1 h. The mixture was poured into a 1:1 mixture of saturated $NaHCO_3$ and 5% $Na_2S_2O_3$ solution, and was extracted with ether. The ethereal solution was washed with brine, dried over Na_2SO_4 and concentrated. The residue was purified with column chromatography (silica gel, AcOEt:hexane, 23:77) to provide 10-(2-[2-(2-azidoethoxy)ethoxy]ethoxy)decanal (1.31 g, 3.98 mmol) as an oil. IR (film) 2930, 2105, 1720, 1460, 1350, 1300, 1125 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (10H, m), 1.64 (4H, m), 2.43 (2H, dt, $J=$ 1.8, 7.4 Hz), 3.41 (2H, brt, $J=5.1$ Hz), 3.46 (2H, t, $J=$ 6.8 Hz), 3.60 (2H, m), 3.68 (8H, m), 9.77 (1H, t, $J=$ 1.8 Hz). MS (CI, NH3) m/z 347 ($M+NH_4^+$). HRMS (FAB) found 330.2398, calcd for $C_{16}H_{32}N_3O_4$ (MH^+) 330.2395.

To a mixture of the aldehyde thus obtained (1.31 g, 3.98 mmol) and 2-methyl-2-butene (2 M in THF, 5.0 mL) in *t*-BuOH (12 mL) was added a mixture of $NaClO_2$ (983 mg, 5.17 mmol) and NaH_2PO_4 (1.0 g) in H_2O (5.0 mL). The mixture was stirred for 30 min and then THF and *t*-BuOH were removed in vacuo at <20 °C. The residue was diluted with 1% NaOH and washed with ether. The aq layer was acidified to pH 3 with 2 M HCl and extracted with AcOEt. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated to give pure 10-(2-[2-(2-azidoethoxy)-ethoxy]ethoxy)decanoic acid (1.25 g, 3.62 mmol, 91%) as an oil. IR (film) 3200, 2930, 2860, 2105, 1735, 1710, 1460, 1290, 1120 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.31 (10H, m), 1.60 (4H, m), 2.36 (2H, t, $J=7.5$ Hz), 3.41 (2H, brt, $J=4.8$ Hz), 3.47 (2H, t, $J=6.6$ Hz), 3.60 (2H, m), 3.69 (8H, m). MS (EI) m/z (%) 346 (7.0, MH^+), 328 (5.0, $[M^+-H_2O]$), 259 (25, $[M^+-N_3CH_2CH_2O]$), 215 (26, $[M^+-N_3CH_2CH_2OCH_2CH_2O]$), 160 (base peak). HRMS (EI) found 346.2347, calcd for $C_{16}H_{31}N_3O_5$ (MH^+) 346.2344.

A mixture of the carboxylic acid thus prepared (1.20 g, 3.47 mmol), HOBT (700 mg, 4.58 mmol), (L)-tyrosine methyl ester **4** (900 mg, 4.61 mmol), Et_3N (1.0 mL, 720 mg, 7.13 mmol), and WSCI (850 mg, 4.45 mmol) in CH_2Cl_2 (10.0 mL) was stirred for 18 h. The mixture was poured into 2 M HCl and extracted with AcOEt. The organic layer was washed with brine, dried over Na_2SO_4 and concentrated. The crude material was purified with column chromatography (silica gel, acetone:CH_2Cl_2, 6:94) to give **5** (1.55 g, 2.96 mmol, 94%) as an oil. $[\alpha]_D^{20}$ $+47.9°$ (*c* 1.04, $CHCl_3$). IR (film) 3315, 2930, 2860, 2105, 1740, 1655, 1510, 1440, 1225, 1125, 830 cm^{-1}. ^1H NMR (300 MHz, $CDCl_3$) δ 1.30 (10H, m), 1.59 (4H, m), 2.19 (1H, dt, $J=7.2$, 14.4 Hz), 2.22 (1H, dt, $J=7.2$, 14.4 Hz), 2.99 (1H, dd, $J=6.3$, 14.1 Hz), 3.12 (1H, dd, $J=5.4$, 14.1 Hz), 3.38 (2H, t, $J=5.2$ Hz), 3.50 (2H, t, $J=6.3$ Hz), 3.65 (10H, m), 3.77 (3H, s), 4.86 (1H, ddd, $J=5.4$, 6.3, 10.8 Hz), 5.91 (1H, brd, $J=10.8$ Hz), 6.77, and 6.94 (each 2H, brd, $J=8.7$ Hz). MS (EI) m/z (%) 523 (1.0, MH^+), 491 (0.5, $[M^+$-MeO]), 463 (1.1, $[M^+$-MeO-N_2]), 345 (40, $[M^+-HOC_6H_4CH_2CHCO_2Me]$), 196 (22, [Tyr-$OMe^+$ + H]), 178 (73, [TyrOMe^+-NH_3]), 107 (100, $HOC_6H_4CH_2^+$). HRMS (FAB) found 523.3123, calcd for $C_{26}H_{43}N_4O_7$(MH^+) 523.3134.

Bio-C_{10}-Tyr-OMe (6). A suspension of **5** (98.0 mg, 187 μmol) and 20% $Pd(OH)_2$/C (10 mg) in MeOH (5.0 mL) was stirred vigorously under H_2 atmosphere for 1 h. After removal of the catalyst by filtration through a pad of Celite, the filtrate was concentrated to give the amine in almost pure form. The obtained amine was diluted with CH_2Cl_2 (3.0 mL) and THF (3.0 mL), and was stirred with (+)-biotin *p*-nitrophenyl ester (71.3 mg, 195 μmol) and Et_3N (40 mL) for 10 h. The mixture was concentrated and the residue was purified with column chromatography (silica gel, MeOH:AcOEt, 10:90) to give **6** (125 mg, 173 μmol, 93%) as an oil. $[\alpha]_D^{20}$ $+29.9°$ (*c* 1.26, MeOH). IR (film) 3280, 2930, 2860, 1690, 1655, 1545, 1520, 1460, 1245, 1105, 830 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.30 (10, m), 1.60 (10H, m), 2.18 (2H, t, $J=7.6$ Hz), 2.24 (2H, t, $J=$ 7.6 Hz), 2.73 (1H, brd, $J=13.6$ Hz), 2.88 (1H, dd, $J=8.8$, 13.6), 2.94 (1H, dd, $J=5.2$, 13.6), 3.07 (1H, dd, $J=5.6$, 13.6 Hz), 3.23 (1H, ddd, $J=4.4$, 6.0, 9.2 Hz), 3.38 (2H, brq, $J=5.2$ Hz), 3.49 (2H, t, $J=6.8$ Hz), 3.56 (2H, t, $J=5.6$ Hz), 3.60 (2H, m), 3.65 (6H, m), 3.71 (3H, s), 4.32 (1H, dd, $J=4.4$, 8.0 Hz), 4.51 (1H, dd, $J=5.2$, 8.0 Hz), 4.62 (1H, m), 6.72, 7.03 (each 2H, brd, $J=$ 8.4 Hz), 8.00 (1H, brt, $J=5.2$ Hz), 8.19 (1H, brd, $J=$ 8.0 Hz). MS (CI, NH_3) m/z 723 (MH^+), 740 ($M+$ NH_4^+). HRMS (FAB) found 723.4005, calcd for $C_{36}H_{59}N_4O_9S$ (MH^+) 723.4006.

Bio-C_{10}-PhTX343 (7). A solution of **6** (125 mg, 173 μmol) in a mixture of MeOH (1.0 mL) and aq NaOH (15 mg, 375 μmol, in 1.0 mL) was stirred for 4 h. After MeOH was removed in vacuo at <20 °C, the mixture was acidified to pH 2 by aqueous HCl and was kept at 0 °C for 10 h. The fine crystalline product was collected as the corresponding carboxylic acid in almost pure form (92.0 mg, 129 μmol, 75%). Mp 103–105 °C (fine needles, from H_2O). $[\alpha]_D^{20}$ $+4.95°$ (*c* 0.75, MeOH). IR (KBr) 3300, 2920, 2850, 1720, 1640, 1535, 1240, 1100 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.30 (10H, m), 1.60 (10H, m), 2.17 (2H, t, $J=7.6$ Hz), 2.24 (2H, t, $J=7.2$ Hz), 2.73 (1H, d, $J=12.8$ Hz), 2.87 (1H, dd, $J=9.2$, 14.0 Hz), 2.94 (1H, dd, $J=4.8$, 12.8 Hz), 3.13 (1H, dd, $J=4.8$, 14.0 Hz), 3,22 (1H, m), 3.37 (2H, q, $J=5.2$ Hz), 3.50 (2H, t, $J=6.8$ Hz), 3.56 (2H, t, $J=$ 5.6 Hz), 3.61 (2H, m), 3.66 (6H, m), 4.32 (1H, dd, $J=4.4$, 7.6 Hz), 4.51 (1H, dd, $J=4.8$, 7.6 Hz), 4.62 (1H, b), 6.71, 7.06 (each 2H, brd, $J=8.4$ Hz), 8.01 (1H, br). MS (CI, NH_3) m/z 726 ($M+NH_4^+$), 709 (MH^+), 691 (MH^+-H_2O). HRMS (FAB) found 709.3842, calcd for $C_{35}H_{56}N_4O_9S$ (MH^+) 709.3849.

A mixture of the above carboxylic acid (16.0 mg, 22.6 μmol) and *p*-nitrophenol (4.0 mg, 28.7 μmol) in THF (1.0 mL) was stirred with DCC (6.2 mg, 30.1 μmol) for 12 h, then a solution of spermine (12 mg, 59 μmol) in THF (1.0 mL) was added and the whole mixture was stirred for another 2 h. The mixture was adsorbed on silica gel column intact. Successive elution with MeOH and *i*-PrOH:MeOH (5:95) gave *p*-nitrophenol and **7** (12.0 mg, 13.7 μmol) as an oil, respectively. $[\alpha]_D^{20}$ +19.3° (*c* 1.05, MeOH). IR (film) 3280, 2930, 1690, 1655, 1560, 1545, 1250, 1106 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 1.30 (10H, m), 1.60 (28H, m), 2.20 (2H, t, *J* = 7.2 Hz), 2.24 (2H, t, *J* = 7.2 Hz), 2.52 (2H. t, *J* = 7.2 Hz), 2.60 (2H, t, *J* = 6.8 Hz), 2.67 (2H, t, *J* = 6.8 Hz), 2.72 (1H, d, *J* = 12.4 Hz), 2.76 (2H, t, *J* = 7.4 Hz), 2.81 (1H, dd, *J* = 8.0, 13.6 Hz), 2.94 (1H, dd, *J* = 4.4, 12.4 Hz), 2.98 (1H, dd, *J* = 7.2, 13.6 Hz), 3.16 (1H, dd, *J* = 6.8, 13.6 Hz), 3.23 (1H, m), 3.24 (1H, dd, *J* = 6.8, 13.6 Hz), 3.81 (2H, t, *J* = 5.6 Hz), 3.50 (2H, t, *J* = 6.4 Hz), 3.56 (2H, t, *J* = 5.6 Hz), 3.61 (2H, m), 3.67 (6H, m), 4.32 (1H, dd, *J* = 4.4, 8.0 Hz), 4.49 (1H, dd, *J* = 7.2, 8.0 Hz), 4.51 (1H, ddd, *J* = 0.4, 4.4, 8.0 Hz), 6.71, 7.06 (each 2H, brd, *J* = 8.4 Hz). MS (CI, NH_3) *m/z* 893 (MH^+), 875 (MH^+-H_2O), 836 (MH^+-$CH_2{=}CHCH_2NH_2$). HRMS (FAB) found 893.5878, calcd for $C_{45}H_{80}N_8O_8S$ (MH^+), 893.5903.

Bio-C_{10}-PhTX343-Lys (8). Compound **7** (9.6 mg, 10.8 μmol) was stirred with α,ε-bis-Boc-(L)-lysine *N*-hydroxysuccinimide ester (4.9 mg, 11.3 μmol) in DMF (1.0 mL) for 2 h. The mixture was purified by column chromatography (silica gel, *i*-$PrNH_2$:MeOH, 7:93) to give the coupling product (12.5 mg, 10.2 μmol, 95%) as an oil. ^1H NMR (400 MHz, CD_3OD) δ 1.31 (12H, m), 1.46, 1.47 (each 9H, s), 1.6 (22H, m), 2.20 (2H, t, *J* = 7.2 Hz), 2.24 (2H, t, *J* = 7.2 Hz), 2.53 (2H, t, *J* = 7.6 Hz), 2.64 (6H. m), 2.72 (1H, d, *J* = 12.4 Hz), 2.82 (1H, dd, *J* = 8.4, 13.6 Hz), 2.94 (1H, dd, *J* = 5.2, 12.4 Hz), 2.98 (1H, dd, *J* = 7.2, 13.6 Hz), 3.05 (2H, t, *J* = 6.4 Hz), 3.23 (5H, m), 3.38 (2H, t, *J* = 5.6 Hz), 3.50 (2H, t, *J* = 7.2 Hz), 3.56 (2H, t, *J* = 5.2 Hz), 3.61 (2H, m), 3.66 (6H, m), 3.96 (1H, br), 4.32 (1H, dd, *J* = 4.4, 7.6 Hz), 4.49 (7.2, 8.4 Hz), 4.51 (1H, dd, *J* = 5.2, 7.6 Hz), 6.72, 7.07 (each 2H, d, *J* = 8.4 Hz)

A solution of the above adduct (12.5 mg, 10.2 μmol) in a mixture of PhSH (20 μL) and TFA (1.0 mL) was stirred for 1 h. After concentration, the residue was purified with Amberlite IRA410 (OH^- form, eluted with H_2O) to give the product in free amine state with small amount of phenyl disulfide. Further purification with column chromatography (silica gel, *i*$PrNH_2$:MeOH, 15:85) afforded compound **8** (6.5 mg, 6.3 μmol, 62%) as an oil. ^1H NMR (400 MHz, CD_3OD) δ 1.31 (12H, m), 1.6 (22H, m) 2.20 (2H, t, *J* = 7.2 Hz), 2.24 (2H, t, *J* = 7.2 Hz), 2.53 (2H, t, *J* = 7.2 Hz), 2.65 (6H, m), 2.73 (2H, t, *J* = 7.2 Hz), 2.73 (1H, d, 12.8 Hz), 2.81 (1H, dd, *J* = 8.0, 14.0 Hz), 2.94 (1H, dd, *J* = 4.8, 12.8 Hz), 2.99 (1H, dd, *J* = 7.2, 14.0 Hz), 3.2 (4H, m), 3.29 (2H, t, *J* = 6.8 Hz), 3.38 (2H, t, *J* = 5.6 Hz), 3.50 (2H, t, *J* = 6.8 Hz), 3.57 (2H, t, *J* = 5.6 Hz), 3.61 (2H, m), 3.67 (6H, m), 6.72, 7.06 (each 2H, d, *J* = 8.1 Hz), MS (CI, NH_3) *m/z* 1021 (MH^+), 1002 (M^+-H_2O), 893 (MH^+-Lys). HRMS (FAB) found 1021.6849, calcd for $C_{51}H_{92}N_{10}O_9S$ (MH^+) 1021.6854.

***N*-10-[2-(2-[2-((β-Sulfoxybiotinylamino)ethoxy]ethoxy)-ethoxy]decanoyl-(L)-tyrosine (9β).** With similar treatment as mentioned above in the preparation of **7**, hydrolysis of **6** afforded the corresponding carboxylic acid. A suspension of the obtained carboxylic acid (33.0 mg, 46.6 μmol) and $NaIO_4$ (11 mg, 51.4 μmol) in a mixture of MeOH (2.0 mL) and H_2O (400 μL) was stirred for 10 h. After MeOH was removed at < 20 °C, the mixture was poured into brine (10 mL) containing HCl (2 M, 50 μL) and $Na_2S_2O_3$ (saturated, 20 μL), and was extracted with *n*-BuOH. The organic solution was dried over $MgSO_4$ and concentrated to give **9**β. ^1H NMR (400 MHz, $CDCl_3$) δ 1.29 (10 H, m), 1.55 (6H, m), 1.72 (2H, quint, *J* = 7.5 Hz), 1.82 (2H, m), 2.16, 2.21 (each 1H, dt, *J* = 7.4, 14.1 Hz), 2.28 (2H, t, *J* = 7.2 Hz), 2.87 (1H, dd, *J* = 8.9, 13.9 Hz), 3.12 (1H, dd, *J* = 6.4, 13.9 Hz), 3.27 (2H, m), 3.39 (2H, t, *J* = 5.4 Hz)m 3.49 (2H, t, *J* = 6.6 Hz), 3.60 (m, 12H), 4.51 (1H, brd, *J* = 4.6, 8.5 Hz), 4.61 (1H, dd, *J* = 5.3, 8.9 Hz), 4.72 (1H, ddd, *J* = 2.2, 6.4, 8.9 Hz), 6.70, 7.03 (each 2H, brd, *J* = 8.5 Hz).

β(O)-Bio-C_{10}-PhTX343 (10β). The coupling between **9**β and spermine was carried out using *p*-nitrophenol and DCC analogously to the preparation of **7**, affording **10**β (66%) as an oil. $[\alpha]_D^{20}$ +6.9° (*c* 0.670, MeOH). IR (KBr) 3280, 2930, 1685, 1655, 1460, 1200 cm^{-1}. ^1H NMR (400 MHz, CD_3OD), δ 1.30 (10H, m), 1.60 (28H, m), 2.16, 2.21 (each 1H, dt, *J* = 7.4, 14.1 Hz), 2.28 (2H, t, *J* = 7.2 Hz), 2.56 (2H, t, *J* = 7.2 Hz), 2.60–2.85 (13H, m), 2.98 (1H, dd, *J* = 7.0, 13.7 Hz), 3.09 (1H, dd, *J* = 6.5, 13.8 Hz), 3.20 (4H, m), 3.39 (2H, t, *J* = 5.6 Hz), 3.50 (2H, t, *J* = 6.6 Hz), 3.54 (1H, dd, *J* = 2.2, 13.8 Hz), 3.6 (12H, m), 4.47 (1H, t, *J* = 7.2 Hz), 4.61 (1H, dd, *J* = 5.3, 8.8 Hz), 4.70 (1H, ddd, *J* = 2.5, 6.5. 8.8 Hz), 6.71, 7.06 (each 2H, brd, *J* = 8.4 Hz). MS (FAB) *m/z* 909 (MH^+), 852 (MH^+-$CH_2{=}CHCH_2NH_2$). HRMS (FAB) found 909.5844, calcd for $C_{45}H_{80}N_8O_9S$ (MH^+) 909.5852.

α(O)-Bio-C_{10}-PhTX343 (10β). A solution of **6** (38.0 mg, 52.6 mol) in CH_2Cl_2 (1.0 mL) was stirred with methyl chloroformate (5 μL, 6.1 mg, 65.0 μmol) in the presence of pyridine (10 μL, 9.8 mg, 123 μmol) for 3 h. The mixture was poured into brine and extracted with AcOEt. The organic solution was dried over $MgSO_4$ and concentrated. The residue was purified by column chromatography (silica gel, MeOH:CH_2Cl_2, 10:90) to give the corresponding (methoxycarbonyl)tyrosine methyl ester (25 mg, 32.1 μmol, 59%) as fine needles. Mp 95–96 °C (from AcOEt-hexane). $[\alpha]_D^{22}$ +49.9° (*c* 1.00, $CHCl_3$). IR (KBr) 3300, 2930, 2860, 1710, 1650, 1545, 1280, 1125 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.30 (10H, m), 1.45 (2H, m), 1.58 (4H, m), 1.70 (4H, m), 2.22 (4H, m), 2.75 (1H, d, *J* = 12.8 Hz), 2.92 (1H, dd, *J* = 4.4, 12.8 Hz), 3.10 (1H, dd, *J* = 6.4, 14.0 Hz), 3.15 (1H, dd, *J* = 6.4, 14.0 Hz), 3.16 (1H, m), 3.46 (4H, m), 3.60 (4H, m), 3.65 (6H, m), 3.74 (3H, s), 3.92 (3H, s), 4.33 (1H, dd, *J* = 4.4, 7.6 Hz), 4.52 (1H, dd, *J* = 5.2, 7.6 Hz), 4.88 (1H, ddd, *J* = 6.4, 6.6, 8.0 Hz), 5.34 (1H, br), 6.40 (1H, brd, *J* = 7.6 Hz), 6.76 (1H, brt, *J* = 5.2 Hz), 7.12 (4H, m). MS

(FAB) *m/z* 781 (MH^+), 803 ($M+Na^+$). HRMS (FAB) found 803.3883, calcd for $C_{38}H_{60}N_4O_{11}SNa$ ($M+Na^+$), 803.3876.

A solution of the ester prepared above (45.0 mg, 57.7 μmol) in MeOH (2.0 mL) was stirred with *N*-bromosuccinimide (NBS) (12.0 mg, 67.4 μmol) for 12 h. After concentration, the residue was purified with column chromatography (silica gel, MeOH:CH_2Cl_2, 10:90) to give the corresponding α-sulfoxybiotinyl ester (39.0 mg, 48.9 μmol, 85%) as an oil. $[\alpha]_D^{22}$ −11.6° (*c* 1.15, MeOH). IR (film) 3315, 2930, 2855, 2472, 1750, 1700, 1685, 1655, 1640, 1460 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.30 (10H, m), 1.54 (2H, quint, *J* = 7.2 Hz), 1.59 (2H, quint, *J* = 6.8 Hz), 1.65 (2H, m), 1.77 (2H, m), 1.96 (1H, m), 2.15 (1H, m), 2.18 (2H, t, *J* = 7.2 Hz), 2.29, 2.31 (each 1H, dt, *J* = 14.0, 7.2 Hz), 2.86 (1H, dd, *J* = 9.2, 6.0 Hz), 2.99 (1H, dd, *J* = 9.2, 14.0 Hz), 3.00 (1H, ddd, *J* = 0.6, 6.8, 16.0 Hz), 3.20 (1H, dd, *J* = 5.4, 14.0 Hz), 3.39 (2H, t, *J* = 5.6 Hz), 3.40 (1H, d, *J* = 16.0 Hz), 3.49 (2H, t, *J* = 6.0 Hz), 3.57 (2H, t, *J* = 5.6 Hz), 3.60 (2H, m), 3.65 (6H, m), 3.73 (3H, s), 3.88 (3H, m), 4.71 (1H, dd, *J* = 5.4, 9.2 Hz), 4.73 (1H, dd, *J* = 6.0, 8.4 Hz), 4.86 (1H, dd, *J* = 6.8, 8.4 Hz), 7.12, 7.26 (each 2H, brs, *J* = 8.8 Hz). MS (FAB) *m/z* 819 ($M+Na^+$), 797 (MH^+). HRMS (FAB) found 819.3857, calcd for $C_{38}H_{60}N_4O_{12}SNa$ ($M+Na^+$) 819.3825.

The α-sulfoxybiotinyl ester prepared above (39 mg, 48.9 μmol) and NaOH (7.0 mg, 175 μmol) was stirred in a mixture of MeOH (2.0 mL) and H_2O (300 μL) for 6 h. After MeOH was removed at < 20 °C, the mixture was poured into brine (10 mL) containing 2 M HCl (30 μL) and extracted with *n*-BuOH. The organic solution was dried with $MgSO_4$, concentrated and the residue was diluted with DMF (2.0 mL). This DMF solution was treated with *p*-nitrophenol, DCC, and then spermine analogously to preparation of **7** to give **10α** (25.0 mg, 38.0 μmol, 62%) as an oil. $[\alpha]_D^{22}$ −2.2° (*c* 0.600, MeOH). IR (KBr) 3280, 2930, 1655, 1565, 1462 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.30 (10H, m), 1.60 (26H, m), 1.97 (1H, m), 2.18 (1H, m), 2.20 (2H, t, *J* = 7.2 Hz), 2.27, 2.33 (each 1H, dt, *J* = 7.4, 14.1 Hz), 2.51 (2H, t, *J* = 7.3 Hz), 2.61 (2H, t, *J* = 6.4 Hz), 2.62-2.73 (6H, m), 2.74 (2H, t, *J* = 7.2 Hz), 2.81 (1H, dd, *J* = 8.4, 13.6 Hz), 2.88 (2H, m), 2.98 (1H, t, *J* = 6.6, 13.2 Hz), 3.02 (1H, t, *J* = 6.7, 13.2 Hz), 3.19, 3.22 (each 1H, m), 3.39 (2H, t, *J* = 5.8 Hz), 3.42 (1H, d, *J* = 13.2 Hz), 3.49 (2H, t, *J* = 6.6 Hz), 3.58 (2H, t, *J* = 5.5 Hz), 3.63 (6H, m), 4.49 (1H, t, *J* = 6.6 Hz), 4.73 (1H, dd, *J* = 6.0, 8.4 Hz), 4.86 (1H, dd, *J* = 6.7, 8.4 Hz), 6.71, 7.06 (each 2H, brd, *J* = 8.4 Hz). MS (FAB) *m/z* 909 (MH^+). HRMS (FAB) found 909.5858, calcd for $C_{45}H_{80}N_8O_9S$ (MH^+) 909.5852.

***N*-10-(2-[2-(2-*N*-Boc-Aminoethoxy)ethoxy]ethoxy)decanoyl-3,5-diiodo-(L)-tyrosine methyl ester (11).** Compound **5** (100 mg, 191 μmol) was hydrogenated by 20% $Pd(OH)_2/C$ (10 mg) analogously to the preparation of **6**. The crude product was diluted with CH_2Cl_2 (2.0 mL), Boc_2O (44.0 mg, 200 μmol) was added, and the whole mixture was further stirred for 12 h. After concentration, the residue was purified by column chromatography (silica gel, acetone:CH_2Cl_2) to give the Boc protected amine (108 mg, 181 μmol, 95%) as an oil. ^1H NMR (400 MHz, $CDCl_3$) δ 1.28 (10 H, m), 1.47 (9H, s), 1.59 (4H, m), 2.14 (1H, dd, *J* = 7.2, 14.4 Hz), 2.22 (1H, dd, *J* = 6.8, 14.4 Hz), 3.01 (1H, dd, *J* = 6.4, 14.4 Hz), 3.14 (1H, dd, *J* = 5.6, 14.4 Hz), 3.54 (4H, m), 3.66 (8H, m), 3.78 (3H, s), 4.88 (1H, dt, *J* = 6.4, 5.6 Hz), 5.1 (1H, br), 5.86 (1H, brd, *J* = 8.0 Hz). 6.78, 6.95 (each 2H, *J* = 8.1 Hz). MS (CI, NH_3) *m/z* 614 ($M+NH_4^+$), 597 (MH^+), 540 (MH^+-$C(CH_3)_3$), 496 (M^+-Boc). HRMS (FAB) found 619.3587, calcd for $C_{31}H_{53}N_2O_9$ (MH^+) 597.3753.

To a suspension of the Boc protected amine (40.0 mg, 67.1 μmol) in MeOH (3.0 mL), $NaHCO_3$ (36 mg, 428 μmol in 1.0 mL H_2O) and iodine (70.0 mg, 275 μmol in 1.0 mL MeOH) was added successively. After stirring vigorously for 30 min, 5% aq $Na_2S_2O_3$ (2.0 mL) was added to the mixture to decompose excess iodine. The mixture was poured into water and extracted with AcOEt. The organic solution was washed with brine, dried over $MgSO_4$ and concentrated. Purification by column chromatography (silica gel, acetone:CH_2Cl_2) gave **11** (55.4 mg, 65.3 μmol, 97%) as an oil. IR (film) 3320, 2930, 2860, 1745, 1710, 1680, 1650, 1540, 1460, 1365, 1275, 1250, 1170, 1125 cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$) δ 1.32 (10H, m), 1.47 (9H, s), 1.62 (4H, m), 2.20 (1H, dd, *J* = 7.2, 14.8 Hz), 2.25 (1H, dd, *J* = 7.6, 14.8 Hz), 2.97 (1H, d, *J* = 5.6, 14.0 Hz), 3.07 (1H, dd, *J* = 5.6, 14.0 Hz), 3.33 (2H, bq, *J* = 5.2 Hz), 3.48 (2H, t, *J* = 6.8 Hz), 3.56 (2H, t, *J* = 5.2 Hz), 3.62 (2H, m), 3.66 (6H, m), 3.78 (3H, s), 4.82 (1H, dt, *J* = 7.6, 5.6 Hz), 5.06 (1H, br), 5.82 (1H, brs), 5.97 (1H, brd, *J* = 7.6 Hz), 7.44 (2H, s). MS (CI, NH_3) *m/z* 866 ($M+NH_4^+$), 849 (MH^+), 848 (M^+).

***N*-10-[2-(2-[2-(Biotinylamino)ethoxy]ethoxy)ethoxy]decanoyl-(L)-(3,5-diiodo)tyrosine methyl ester (12).** A solution of **11** (58.0 mg, 68.8 μmol) in a mixture of PhSH (20 μL) and TFA (1.0 mL) was stirred for 1 h. The mixture was concentrated and then diluted with CH_2Cl_2 (1.0 mL). Biotin *p*-nitrophenyl ester (28 mg, 76.7 μmol) was then added and the mixture was stirred for 6 h, washed with brine, dried over Na_2SO_4, and concentrated. Purification with column chromatography (silica gel, MeOH:CH_2Cl_2, 7:93) afforded **12** (58.0 mg, 59.4 μmol, 86%) as an oil. IR (film) 3290, 2930, 2860, 1730, 1695, 1645, 1555, 1540, 1240, 1205, 1130 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.31 (10H, m), 1.6 (10H, m), 2.18 (1H, dd, *J* = 6.8, 14.0 Hz), 2.22 (1H, dd, *J* = 6.4, 14.0 Hz), 2.24 (2H, t, *J* = 7.6 Hz), 2.73 (1H, *J* = 12.8 Hz), 2.80 (1H, dd, *J* = 10.0, 14.0 Hz), 2.94 (1H, dd, *J* = 4.0, 12.8 Hz), 3.08 (1H, dd, *J* = 5.0, 14.0 Hz), 3.22 (1H, m), 3.38 (2H, t, *J* = 5.2 Hz), 3.50 (2H, t, *J* = 6.8 Hz), 3.57 (2H, t, *J* = 5.6 Hz), 3.61 (2H, m), 3.66 (6H, m), 3.74 (3H, s), 4.32 (1H, dd, *J* = 4.4, 8.0 Hz), 4.51 (1H, dd, *J* = 4.8, 8.0 Hz), 4.63 (1H, dd, *J* = 5.0, 10.0 Hz), 7.61 (2H, s). MS (FAB) *m/z* 975(MH^+). HRMS (FAB) found 975.1920, calcd for $C_{36}H_{57}N_4O_9I_2S$ (MH^+) 975.1930.

Bio-C_{10}-PhTX(I_2)343 (13). A solution of **12** (50 mg, 50.1 μmol) in MeOH (1.0 mL) was stirred with aq NaOH (6.0 mg, in 50 μL) for 3 h. Aq TFA solution was

added into the mixture until acidic, and the mixture was concentrated in vacuo under Ar atmosphere. The residue was purified by Amberlite IRA410. After elution of the impurity by H_2O, subsequent elution with 10% aq AcOH afforded the carboxylic acid as an oil. IR (film) 3280, 2920, 1695, 1645, 1555, 1540, 1455, 1240, 1090 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 1.22 (2H, m), 1.31 (8H, m), 1.6 (10H, m), 2.16 (1H, dd, $J=6.8$, 14.0 Hz), 2.24 (1H, dd, $J=6.4$, 14.0 Hz), 2.24 (2H, t, $J=7.5$ Hz), 2.73 (1H, $J=13.0$ Hz), 2.82 (1H, dd, $J=9.6$, 14.0 Hz), 2.95 (1H, dd, $J=5.1$, 13.0 Hz), 3.13 (1H, dd, $J=4.7$, 14.0 Hz), 3.23 (1H, m), 3.38 (2H, t, $J=5.0$ Hz), 3.49 (2H, t, $J=6.5$ Hz), 3.57 (2H, t, $J=5.5$ Hz), 3.61 (2H, m), 3.66 (6H, m), 3.74 (3H, s), 4.34 (1H, dd, $J=4.4$, 7.6 Hz), 4.52 (1H, dd, $J=5.1$, 7.6 Hz), 4.62 (1H, dd, $J=4.7$, 9.6 Hz), 7.62 (2H, s). MS (FAB) m/z 983 ($M+Na^+$), 961 (MH^+). HRMS (FAB) found 961.1818, calcd for $C_{35}H_{55}N_4O_9I_2S$ (MH^+), 961.1773.

The carboxylic acid obtained above (40 mg, 41.7 μmol) was coupled with spermine using *p*-nitrophenol and DCC analogously to the preparation of **7**, affording **13** (20 mg, 17.5 μmol, 42%) as a caramel. IR (film) 3280, 2920, 2860, 1695, 1680, 1660, 1645, 1555, 1455, 1305, 1120 cm^{-1}. ^1H NMR (500 MHz, CD_3OD) δ 1.35 (10H, m), 1.60 (8H, m), 1.92 (2H, quint, $J=7.5$ Hz), 2.24 (4H, m), 2.47 (2H, m), 2.68 (1H, t, $J=6.1$ Hz), 2.73 (1H, d, $J=12.5$ Hz), 2.75 (1H, dd, $J=9.3$, 13.5 Hz), 2.78 (1H, dd, $J=6.0$, 13.5 Hz), 2.84 (2H, t, $J=6.5$ Hz), 2.93 (6H, m), 3.23 (1H, m), 3.38 (2H, t, $J=5.5$ Hz), 3.40 (1H), 3.49 (2H, t, $J=6.5$ Hz), 3.57 (2H, t, $J=5.5$ Hz), 3.59 (2H, m), 3.64 (6H, m), 4.33 (1H, dd, $J=4.5$, 7.9), 4.35 (1H, dd, $J=6.1$, 9.3 Hz), 5.51 (1H, dd, $J=4.5$, 7.6 Hz), 7.49 (2H, s). MS (FAB) 1167 ($M+Na^+$), 1145 (MH^+). HRMS (FAB) found 1145.3875, calcd for $C_{45}H_{79}N_8O_8I_2S$ (MH^+) 1145.3827.

Bio-C_{10}-PhTX(I_2)-343-Lys (14). A solution of **12** (12.0 mg, 10.4 μmol) in DMF (1.0 mL) was stirred with α,ε-bis-*N*-Boc-(L)-lysine *N*-hydroxysuccinimide ester (4.8 mg, 10.8 μmol) for 2 h. Column chromatography (silica gel, *i*-$PrNH_2$:MeOH, 3:97) gave the adduct (15.0 mg, 10.2 μmol, 92%) as an oil. IR (film) 3280, 2920, 2850, 1695, 1650, 1540, 1455, 1250, 1170, 1120 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.35 (10H, m), 1.56, 1.47 (each 9H, s), 1.50 (4H, m), 1.65 (16H, m), 1.95 (2H, quint, $J=7.2$ Hz), 2.23 (1H, dd, $J=7.6$, 14.0 Hz), 2.25 (2H, t, $J=7.2$ Hz), 2.27 (1H, dd, $J=7.6$, 14.0 Hz), 2.46 (2H, m), 2.68 (2H, t, $J=6.0$ Hz), 2.74 (1H, d, $J=12.8$ Hz), 2.74 (1H, dd, $J=9.6$, 13.2 Hz), 2.77 (1H, dd, $J=5.2$, 13.2 Hz), 2.91 (5H, m), 2.96 (1H, dd, $J=4.8$, 12.8 Hz), 3.05 (2H, t, $J=6.8$ Hz), 3.22 (1H, m), 3.38 (2H, t, $J=5.6$ Hz), 3.41 (1H), 3.49 (2H, t, $J=6.8$ Hz), 3.57 (2H, t, $J=5.6$ Hz), 3.59 (2H, m), 3.65 (6H, m), 3.97 (1H, brd, $J=4.4$, 6.0 Hz), 4.33 (1H, dd, $J=4.4$, 7.6 Hz), 4.37 (1H, dd, $J=6.0$, 9.6 Hz), 4.51 (1H, dd, $J=4.4$, 7.6 Hz), 7.50 (2H, s). HRMS (FAB) found 1473.5859, calcd for $C_{61}H_{107}N_{10}O_{13}I_2S$ (MH^+) 1473.5826.

Deprotection of Boc groups was carried out using TFA and PhSH analogously to the preparation of compound **8**. 11.0 mg of the above prepared adduct (6.8 μmol) afforded **14** (5.0 mg, 3.9 μmol, 58%) as an oil. IR (film) 3270, 2930, 1680, 1645, 1555, 1455, 1125 cm^{-1}. ^1H NMR (400 MHz, CD_3OD) δ 1.35 (10H, m), 1.48 (2H, quint, $J=7.0$ Hz), 1.65 (18H, m), 1.95 (2H, quint, $J=7.2$ Hz), 2.23 (1H, dd, $J=7.6$, 14.0 Hz), 2.25 (2H, t, $J=7.2$ Hz), 2.27 (1H, dd, $J=7.6$, 14.0 Hz), 2.49 (2H, m), 2.71 (1H), 2.73 (1H, d, $J=12.5$ Hz), 2.74 (1H, dd, $J=9.5$, 13.0 Hz), 2.78 (1H, dd, $J=6.0$, 13.3 Hz), 2.86 (2H, t, $J=7.5$ Hz), 2.92 (4H, m), 2.94 (1H, dd, $J=4.5$, 12.5 Hz), 3.23 (1H, m), 3.36 (1H, t, $J=7.0$ Hz), 3.38 (2H, t, $J=5.5$ Hz), 3.41 (1H), 3.49 (2H, t, $J=7.0$ Hz), 3.58 (2H, t, $J=6.0$ Hz), 3.60 (2H, m), 3.65 (6H, m), 4.33 (1H, dd, $J=3.0$, 7.5 Hz), 4.36 (1H, dd, $J=6.0$, 9.5 Hz), 4.51 (1H, dd, $J=4.5$, 7.5 Hz), 7.50 (2H, s). HRMS (FAB) found 1245.4591, calcd for $C_{51}H_{91}N_{10}O_9I_2S$ (MH^+) 1245.4600.

β(O)-Bio-C_{10}-PhTX(I_2) 343 (15). A solution of **12** (46.0 mg, 47.2 μmol) in MeOH (1.0 mL) was stirred with aq NaOH (4.0 mg, in 20 μL) under ambient atmosphere for 4 h. Aq TFA solution was added into the mixture until acidic, and the mixture was concentrated. The residue obtained was purified by silica gel column chromatography. Successive elution with AcOH:CH_2Cl_2:MeOH (0.3:50:50) and AcOH:CH_2Cl_2:MeOH (1:50:50) gave the corresponding biotinyl carboxylic acid (12 mg, 12.4 μmol, 26%) and β-sulfoxybiotinyl carboxylic acid (30 mg, 30.7 mmol, 65%) as oil, respectively. ^1H NMR (400 MHz, CD_3OD) δ 1.25 (2H, m), 1.30 (8H, m), 1.58 (6H, m), 1.73 (2H, quint, $J=7.2$ Hz), 1.90 (2H, m), 2.18 (1H, dd, $J=7.2$, 13.6 Hz), 2.22 (1H, dd, $J=8.0$, 13.6 Hz), 2.28 (2H, t, $J=7.2$ Hz), 2.82 (1H, dd, $J=9.2$, 14.0 Hz), 3.10 (1H, d, $J=14.0$ Hz), 3.15 (2H, m), 3.39 (2H, t, $J=5.2$ Hz), 3.50 (2H, t, $J=6.8$ Hz), 3.56 (1H, dd, $J=2.0$, 14.0 Hz), 3.68 (2H, t, $J=5.6$ Hz), 3.60 (2H, m), 3.65 (67H, m), 4.49 (1H, br), 4.63 (1H, dd, $J=$ 5.2, 8.8 Hz), 4.73 (1H, ddd, $J=2.0$, 6.4, 8.8 Hz), 7.62 (2H, s).

Coupling between the β-sulfoxybiotinyl carboxylic acid (30 mg, 30.7 μmol) and spermine was carried out using *p*-nitrophenol and DCC analogously to the preparation of **7**, giving 10.0 mg of **15** (8.6 μmol, 28%) as a caramel. ^1H NMR (400 MHz, CD_3OD) δ 1.35 (10H, m), 1.55–1.75 (14H, m), 1.9 (2H, m), 1.98 (2H, quint, $J=6.8$ Hz), 2.26 (4H, m), 2.54 (2H, m), 2.70–3.20 (13H, m), 3.38 (2H, t, 5.2 Hz), 3.43 (1H), 3.49 (2H, t, $J=6.8$ Hz), 3.5 (1H), 3.68 (2H, t, $J=5.6$ Hz), 3.60 (2H, m), 3.65 (67H, m), 4.37 (1H, 1H, dd, $J=6.2$, 9.5 Hz), 4.61 (1H, dd, $J=$ 5.3, 8.9 Hz), 4.70 (1H, ddd, $J=2.2$, 6.5, 8.9 Hz), 7.52 (2H, s).

Bio-C_{10}-PhTX343-BPP (16). In darkness, 0.25 M methanolic TFA solution was added into a mixture of **7** (4.40 mg, 4.95 μmol) and BBP aldehyde **19** (1.30 mg, 4.50 μmol) in MeOH (2.0 mL) until neutral. Then $NaBH_3CN$ (310 μg, 4.95 μmol) was added and the mixture was stirred for 12 h and concentrated. The residue was purified by preparative silica gel TLC developed with *i*-$PrNH_2$:MeOH (5:95) to give **16** (4.30 mg, 4.54 μmol, 82%). $R_f=0.5$. ^1H NMR (300 MHz, CD_3OD) α 1.20–1.82 (28H, m), 2.17 (2H, t, $J=8.0$ Hz), 2.21 (2H,

t, $J=7.3$ Hz), 2.53 (2H, t, $J=7.2$ Hz), 2.65 (2H, brt, $J=6.8$ Hz), 2.69–2.83 (8H, m), 2.91 (3H, m), 3.03 (2H, t, $J=5.1$ Hz), 3.20 (8H, m), 3.35 (2H, t, $J=5.4$ Hz), 3.46 (2H, t, $J=6.6$ Hz), 3.53 (2H, t, $J=5.4$ Hz), 3.58 (2H, m), 3.62 (6H, m), 4.22 (2H, t, $J=5.2$ Hz), 4.29 (1H, dd, $J=4.4$, 7.9 Hz), 4.47 (2H, m), 6.69, 7.04 (each 2H, d, $J=8.5$ Hz), 7.18 (1H, brs), 7.69 (1H, brs), 7.91 (1H, t, $J=2.0$ Hz). ^{19}F-NMR (282 MHz, CD_3OD, ppm versus $CFCl_3$) δ −65.24. HRMS (FAB) found 1166.6255, calcd for $C_{55}H_{87}N_{11}F_3O_{11}S$ (MH+) 1166.6274.

Biotin methyl ester (17). A solution of biotin (400 mg, 1.64 mmol) in MeOH (5.0 mL) was stirred with trimethyl-silyldiazomethane (2 M in hexane, 2.0 mL) at 0 °C for 10 h. After concentration, the residue was recrystallized with AcOEt:MeOH to give biotin methyl ester **17** (370 mg, 1.43 mmol, 87%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.45 (2H, quint, $J=7.5$ Hz), 1.67 (4H, m), 2.37 (2H, t, $J=7.4$ Hz), 2.75 (1H, dd, $J=0.8$, 12.7 Hz), 2.94 (1H, dd, $J=5.0$, 12.7 Hz), 3.22 (1H, m), 3.69 (3H, s), 4.32 (1H, dd, $J=4.5$, 7.8 Hz), 4.51 (1H, ddd, $J=0.8$, 5.0, 7.8 Hz).

Biotin β-phenethylamide (18). To a solution of biotin *p*-nitrophenyl ester (200 mg, 547 μmol) in CH_2Cl_2 (2.0 mL) was added β-phenethyl-amine (100 μL, 795 μmol) at 0 °C. After stirring for 30 min, the mixture was poured into saturated $NaHCO_3$ and extracted with AcOEt. The organic solution was washed with 2 M $NaHCO_3$ and brine successively, dried over $MgSO_4$ and concentrated. The residue was purified with column chromatography (silica gel, MeOH:AcOEt, 30:70) to give **18** as amorphous powder (150 mg, 431 μmol, 79%). ^{1}H NMR (400 MHz, CD_3OD) δ 1.40 (2H, m), 1.60 (2H, m), 1.70 (2H, m), 2.18 (2H, t, $J=7.4$ Hz), 2.63 (1H, d, $J=12.8$ Hz), 2.81 (1H, d, $J=7.3$ Hz), 2.95 (1H, dd, $J=4.9$, 12.8 Hz), 3.20 (1H, ddd, $J=4.5$, 5.9, 8.9 Hz), 3.42 (1H, dt, J = 1.5, 7.3 Hz), 4.30 (1H, dd, $J=4.5$, 7.9 Hz), 4.51 (1H, ddd, $J=0.8$, 4.9, 7.9 Hz), 7.25 (5H, m). MS (CI, NH_3), *m*/*z* 365 ($M+NH_4^+$), 348 (MH^+).

α-Sulfoxybiotin methyl ester (17α). Biotin methyl ester **17** (100 mg, 387 μmol) was treated with NBS analogously to the preparation of **10**α. Column chromatography (silica gel, MeOH:CH_2Cl_2, 20:80) afforded **17**α (95 mg, 346 μmol, 90%) as a caramel. ^{1}H NMR (400 MHz, CD_3OD) δ 1.70 (4H, m), 1.95 (1H, m), 2.13 (1H, m), 2.44 (2H, t, $J=7.2$ Hz), 2.87 (1H, m), 3.01 (1H, dd, $J=6.4$, 15.2 Hz), 3.40 (1H, d, $J=15.2$ Hz), 3.62 (3H, s), 4.76 (1H, dd, $J=6.0$, 8.4 Hz), 4.91 (1H, dd, $J=6.4$, 8.4 Hz). β-Isomer were not detected in this ^{1}H NMR spectrum.

α-Sulfoxybiotin β-phenethylamide (18α). Biotinamide **18** (21.3 mg, 61.4 μmol) was treated with NBS analogously to the preparation of **10**α. Column chromatography (silica gel, acetone:MeOH, 50:50) gave **18**α (18.3 mg, 50.4 μmol, 82%) as a caramel (α/β, 16/1, by NMR). ^{1}H NMR (400 MHz, CD_3OD) δ 1.60 (2H, m), 1.73 (2H, m), 1.94 (1H, m), 2.13 (1H, m), 2.25 (1H, dt, $J=2.3$, 7.3 Hz), 2.83 (1H, t, $J=7.3$ Hz), 2.83 (1H, m), 3.00 (1H, dd, $J=6.8$, 15.4 Hz), 3.41 (1H, d, $J=15.4$ Hz), 3.45 (2H, m), 4.72 (1H, dd, $J=5.9$, 8.4 Hz), 4.87 (1H, dd, $J=6.8$, 8.4 Hz), 7.24 (5H, m). MS (CI, NH_3) *m*/*z* 381 ($M+NH_4^+$), 364 (MH^+).

β-Sulfoxybiotin methyl ester (17β). Biotin methyl ester **17** (10.0 mg, 38.7 μmol) was treated with $NaIO_4$ analogously to the preparation of **10**β. Column chromatography (silica gel, MeOH:CH_2Cl_2, 20:80) gave **17**β (9.0 mg, 32.8 μmol, 85%) as a caramel (α:β = 1:4, by NMR). ^{1}H NMR (400 MHz, CD_3OD) δ 1.60 (2H, m), 1.75 (2H, m), 1.92 (2H, m), 2.41 (2H, t, $J=7.4$ Hz), 3.09 (1H, dd, $J=6.5$, 13.1 Hz), 3.17 (1H, m), 3.55 (1H, dd, $J=2.2$, 13.1 Hz), 3.69 (3H, s), 4.61 (1H, dd, $J=5.3$, 8.8 Hz), 4.70 (1H, ddd, $J=2.2$, 6.5, 8.8 Hz).

β-Sulfoxybiotin β-phenethylamide (18β). Biotinamide **18** (21.0 mg, 60.4 μmol) was treated with $NaIO_4$ analogously to the preparation of **10**β. Column chromatography (silica gel, MeOH:CH_2Cl_2, 50:50) gave **18**β (20.0 mg, 55.1 μmol, 91%) as a caramel (α/β = 1/4, by NMR). ^{1}H NMR (400 MHz, CD_3OD) δ 1.43 (2H, m), 1.61 (2H, m), 1.82 (2H, m), 2.21 (2H, t, $J=7.3$ Hz), 2.84 (2H, t, $J=6.9$ Hz), 3.21 (1H, dd, $J=6.5$, 13.5 Hz), 3.23 (1H, dt, $J=5.4$, 7.6 Hz), 3.49 (2H, dt, $J=1.3$, 6.9 Hz), 3.63 (1H, dd, $J=2.4$, 13.5 Hz), 4.64 (1H, dd, $J=5.4$, 9.0 Hz), 4.80 (1H, ddd, $J=2.4$, 6.5, 9.0 Hz), 7.35 (5H, m). MS (CI, NH_3) *m*/*z* 381 ($M+NH_4^+$), 364 (MH^+). In the following procedures, all preparations containing proteins were kept on ice and all operations were carried out at 4 °C whenever possible. Total protein concentrations were carried out using bicinchoninic acid (BCA) assay following Sigma procedure TPRO-562. ^{3}H-TCP was purchased from DuPont NEN.

Tissue preparation and ^{3}H-TCP binding assay. The nAChR enriched membrane was obtained from electroplax of *Torpedo californica* following published protocols.[15,40] Tissue frozen at −78 °C was thawed and minced in buffer (5 mM Tris, 1 mM EDTA, 0.1 mM phenylmethylsulfonyl fluoride (PMSF), 154 mM NaCl) to give a tissue homogenate. The homogenate was centrifuged at 1000×*g* for 10 min and the supernatant obtained was then centrifuged at 35,000×*g* for 60 min. The pellet thus obtained was resuspended in buffer and kept on ice. Each batch of tissue preparation was used in binding assay within a week.

About 75 μg of total protein was added into a mixture of buffer, carbamylcholine (final concentration at 100 μM), ^{3}H-TCP (6 nmol) and ligands (amantadine, chlorpromazine and trifluoperazine and biotin-PhTXs, final concentration ranging from 0.03 μM to 300 μM), to a final volume of 500 μL. Total bound radioactivity was controlled to be less than 5% of the total radioactivity by adjusting the amount of tissue used. After incubation for 30 s at rt, the mixture was filtered through GF/B glass fiber filters (diameter 2.5 cm) presoaked in 0.05% polyethyleneimide. The filter was washed extensively with 0.9% NaCl (3 mL×4 times), immersed in Fisher ScintiVerse scintillation cocktail and counted on a Beckman LS-3801 liquid scintillation counter. Each ligand concentration was tested in triplicate with standard variation at less than 10%. The count per minute (cpm) values were analyzed and plotted to find the IC_{50} value.

Partial purification of enriched membrane by immobilized avidin-biotin-PhTX complex. One mM solution of compound **7** (bio-C_{10}-PhTX343) was incubated with avidin (monomeric) immobilized on 4% cross-linked agarose (Sigma) at 4 °C under end-to-end stirring for 8 h. The suspension was filtered through a filter column, and the filtrate was measured in the HABA assay to determine its concentration of **7**. The concentration difference before and after complexation was used to obtain concentration of **7** on agarose, which is ca. 85 μM. The agarose was washed twice with buffer (0.15 M NaCl, 0.01 M NaH_2PO_4, pH 6.8) and then reequilibrated with 0.01 M NaH_2PO_4, pH 6.8. In the batch operation, a solution of nAChR enriched membrane solubilized in 2% sodium cholate (total protein concentration at 1.5 mg/mL) was incubated with this resin at 4 °C for 8 h. After filtration, the resin was washed twice with 0.01% Triton X-100 in neutral buffer, treated with electrophoresis sample solution and analyzed by 10% polyacrylamide gel electrophoresis. Results showed that bands corresponding to nAChR were enriched by the affinity resin but other proteins were also present. In the column method, 5 mL of resin (wet volume) was packed into a plastic column, the nAChR enriched membrane solubilized in 2% sodium cholate was adsorbed onto the resin and eluted with a phosphate buffer (0.01 M NaH_2PO_4, pH 6.8). The fractions were concentrated under vacuum centrifugation, dialyzed against 0.5 mM Tris buffer (pH 7.2) for 6 h, and were analyzed by 10% polyacrylamide gel electrophoresis. Results showed that nAChR was eluted at 5–6 times of the void volume. The dissociation constant of nAChR on this resin was calculated to be ca. 20 μM under the experimental condition.[43]

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