An Immunotherapeutic Program for the Treatment of Nicotine Addiction: Hapten Design and Synthesis

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People continue to smoke and use tobacco products despite well-established hazardous consequences. The most contributing factor is the addictive nature of nicotine. There is no highly effective treatment for the problem of nicotine dependence. Immunotherapy offers an alternative to conventional approaches. The chemistry necessary for a comprehensive immunopharmacological program is presented. Haptens for the generation of antibodies specific for naturally occurring (*S*)-nicotine, (*S*)- and (*R*)-nornicotine, and the metabolite (*S*)-cotinine were prepared with high optical purity. Preliminary data for antinicotine antibodies are reported.

Nicotine is the most widely used addictive drug in the world. As an alkaloid [(*S*)−(−)-1-methyl-2-(3-pyridyl)-pyrrolidine] derived from tobacco leaves, nicotine is available in several forms such as cigarettes, cigars, pipe tobacco, and chewing tobacco. Hence, the drug is intimately linked with cigarette smoking, the leading preventable cause of death in the United States.[1] Smoking contributes to coronary heart disease, stroke, vascular disease, peptic ulcers, chronic lung diseases and lung cancer, and fetal brain damage and morbidity. Although the dangers of smoking are well-known, people continue to smoke.

A great deal of evidence supports the view that people continue to smoke because of the addictive effects of nicotine.[2] However, since nicotine is legally and widely accessible there is relatively little stigma associated with its use, unlike cocaine, heroin, and other illicit drugs. Although a large percentage of addicted smokers have expressed a desire to stop smoking and most who quit do so without treatment, less than 5% of unaided attempts lead to successful long-term abstinence.[3] Similarly, the two most popular therapies, nicotine gum and transdermal nicotine patches used to slowly wean the user off the drug, have afforded inadequate long-term success rates of <20%.[4] Perhaps because relatively little is known about the specific neuropharmacologic mechanisms underlying nicotine addiction and the response to smoking cessation treatment, no highly effective therapy has been developed.

There is a need to develop a treatment approach to nicotine addiction which does not depend solely on unaided compliance or on the administration of nicotine itself for rehabilitation. One alternative might rely on immunological reagents and the immune system. Recently, we demonstrated the efficacy of immunological strategies with regard to the cocaine abuse problem.[5] The immune-mediated binding of nicotine would impede its passage into the central nervous system and would result in a suppression of its characteristic actions. In particular, we believe the use of both active immunization (immunoconjugate vaccine) and passive immunization (monoclonal antibodies, mAbs) protocols would provide the most beneficial therapy. Herein, we describe the design and synthesis of haptens necessary to establish an immunopharmacologic arsenal to combat nicotine dependence. Significantly, we have developed a comprehensive program aimed at nicotine as the primary target, as well as the secondary targets nornicotine and cotinine.

Results and Discussion

In the past 30 years, a number of reports appeared in the literature which described nicotine haptens and immunoconjugates.[6] One of these haptens was recently used in vaccination studies with rats.[7] Our general approach to the design of haptens for naturally occurring ligands has been to retain the significant structural and

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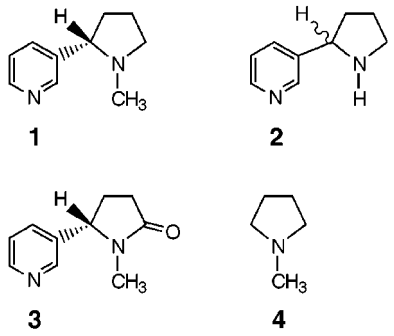

Figure 1. Tobacco components and metabolites under discussion.

stereochemical features of the target compound unaltered upon conversion into a hapten.[5b] Furthermore, the linker required for coupling to a carrier protein should mimic a functional group of the ligand and be of a sufficient length and with a position of attachment to allow appropriate presentation of all important recognition elements. In this way, enhanced antibody titers in vivo, as well as affinity and specificity of isolated mAbs, are obtained that benefit immunopharmacotherapy.

For optimum results, the design and synthesis of nicotine-like haptens demands attention to stereochemistry, acid/base properties, and incorporation of a suitable linker moiety. (*S*)-(−)-Nicotine **1** as well as structurally related compounds, nornicotine **2** present in tobacco and also a metabolite and cotinine **3** the major primary metabolite of nicotine, are included in the development of our program. *N*-Methylpyrrolidine **4**, a minor tobacco component, is only important with regard to the elicitation and selection of premium antinicotine specificities in order to afford the best therapy (Figure 1).

Implementation of our strategy with regard to nicotine requires a hapten with the natural (*S*)-configuration. Although an immune response might be expected against both antipodes of a racemic hapten, stereospecificity and serum titers should be enhanced using the (*S*)-antipode alone. This has been shown for instance in the case of propranolol[8] and in work from our laboratory (unpublished results and see below). The hapten should also contain an alkyl linker of appropriate length at the position of the methyl group on the pyrrolidine nitrogen. With the above approach, all of the characteristics of nicotine remain intact for display to the immune system, namely stereochemistry, two unaltered ring structures, correct charge at physiological pH, and conservation of the important pyrrolidyl methyl group mimicked as part of the linker.

Consequently, hapten **10** (code: **NIC**) was synthesized with the (*S*)-configuration corresponding to natural nicotine (Figure 2). To this end, the formation of 5-bromomyosmine **7** occurred via the base-mediated condensation of ethyl 5-bromonicotinate **6** with *N*-vinylpyrrolidinone, followed by in situ acid-catalyzed hydrolysis of the *β*-keto-*N*-vinyllactam, decarboxylation, and cyclization.[9] The isolated imine was then reduced with sodium borohydride to afford 5-bromonornicotine **8**.

Nornicotine **2b** was then obtained with the (*S*)-(−) stereochemistry in ≥98% ee via a classical resolution of the racemic **8**.[9] In the process, the (*R*)-isomer **2c** was also procured with a similar purity. The reaction with linker **14** proceeded with a good yield of monoalkylation. We anticipated that, after purification of the intermediate benzyl ester, hydrogenation would afford isolated **NIC** ready for use. However, somewhat surprisingly, ∼25% of the ring-opened, 2′-C−N cleavage product **15** was also produced, even at 1 atm of hydrogen and short reaction times, which necessitated purification of the final hapten. Benzylic amines are generally more resistant to hydrogenolysis than benzyl esters and acidic media is often necessary to facilitate the reaction.[10] In this case, the benzylic-like C−N bond adjacent to the pyridyl ring was sufficiently labile to compete with hydrogenolysis of the benzyl ester C−O bond. Although there was no byproduct in the conversion of **8** to nornicotine, the reaction conditions were different and incorporated triethylamine. Since we omitted a base in the reaction of **9** in order to avoid salt formation of the **NIC** hapten, perhaps the more acidic environment, especially after benzyl ester hydrogenolysis, activated the C−N bond toward cleavage.

All previously reported nicotine haptens contained short linkers, at either the pyridyl or pyrrolidyl ring carbons, and were racemic in nature. The linker used here, ∼12 Å in length and with an internal amide bond we recently found useful for hapten immune responses, should yield complete and high affinity recognition of all features of the nicotine molecule.

In the secondary phase of our program, anti-nornicotine and anti-cotinine mAbs provide a potentially beneficial adjunct to nicotine therapy. Nornicotine **2** occurs in tobacco as a mixture of (*R*)- and (*S*)-enantiomeric forms. Although present as only 2% of total alkaloids, the compound is pharmacologically active in the central nervous system similar to nicotine.[11,12] Both optical isomers of nornicotine have similar binding affinities to the nicotinic acetylcholine receptor, but there is evidence that the enantiomers differ in their behavioral and pharmacological activities.[12] Nornicotine also accounts for ∼8% of metabolized nicotine in both humans and rats and has a plasma half-life of about 8 h which is longer than the average 2-h half-life of nicotine.[13] Most significant, animal behavioral studies of rats demonstrate that

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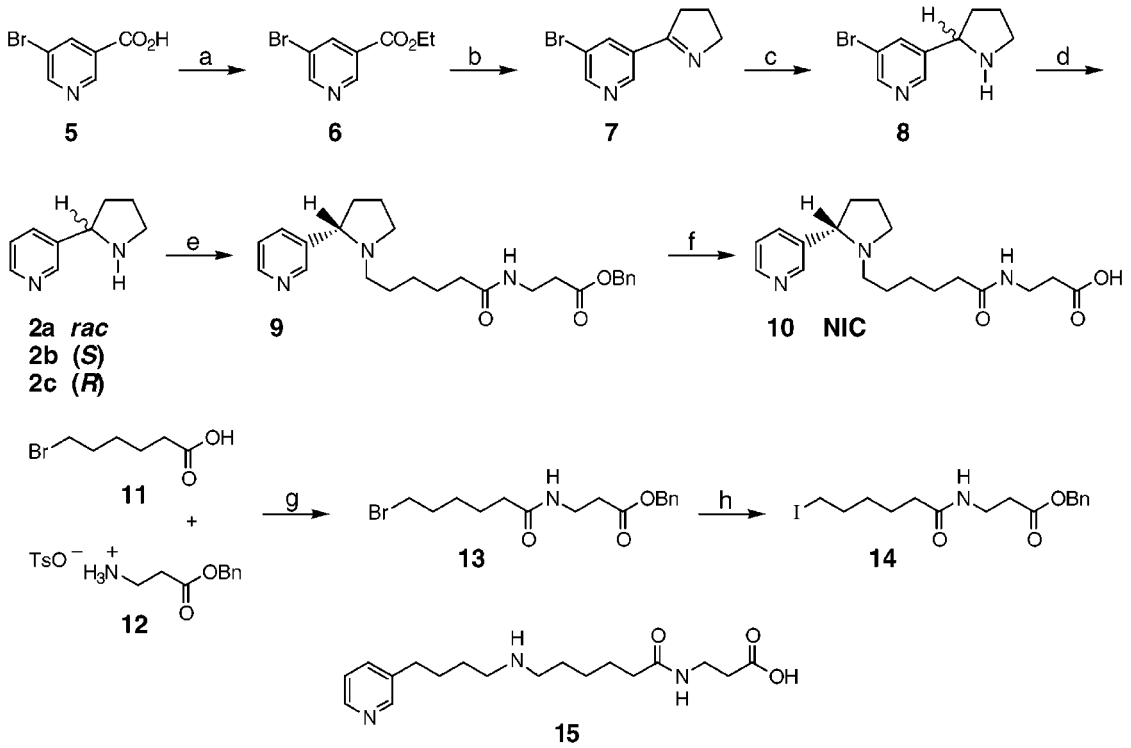

Figure 2. Synthesis of the (*S*)-nicotine hapten: (a) ethanol, concentrated H_2SO_4, reflux; (b) (i) NaH, THF, 1-vinyl-2-pyrrolidinone, reflux, (ii) aq HCl, reflux, (iii) aq NaOH; (c) $NaBH_4$; (d) (i) resolution (MTPA salts), (ii) H_2, Pd/C, NEt_3, EtOH; (e) **14**, DIEA, acetonitrile; (f) H_2, Pd/C, MeOH; (g) HBTU, *N*-methylmorpholine, DMF; (h) NaI, acetonitrile.

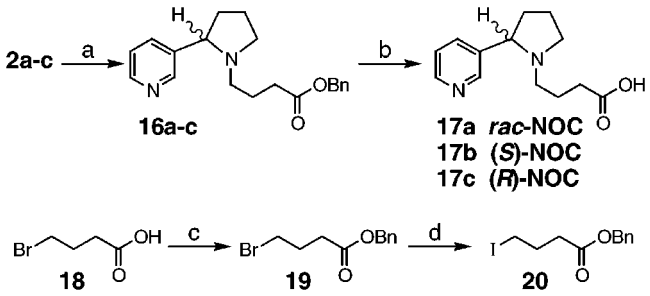

Figure 3. Synthesis of the racemic and (*S*)- and (*R*)-nornicotine haptens: (a) **20**, DIEA, acetonitrile; (b) H_2, Pd/C, MeOH; (c) benzyl alcohol, p-TsOH, cyclohexane; (d) NaI, acetonitrile.

nornicotine effects locomotor activity and is self-administered.[14]

At first, the hapten **16a** (code: **NOC**) was synthesized starting from *rac*-nornicotine **2a** (Figure 3). However, preliminary tests indicated the absence of a viable immune response against the hapten in accord with previous hypotheses. Therefore, separate (*S*)-**NOC** and (*R*)-**NOC** haptens were prepared in order to obtain antibodies specific for each isomer of nornicotine.

Hence, linker **20** was introduced onto the nornicotine framework using reaction chemistry similar to that employed for the preparation of **9**. Hydrogenation of **16a**−**c** afforded **NOC** haptens which required purification, again due to the formation of a ring-opened product analogous to **15**. Although the **NOC** hapten is structurally analogous to **NIC**, the "tuning" of the linker length should be critical. We anticipate that the shorter and less flexible linker will afford a substantial fraction of antibodies that recognize only the pyridyl and pyrrolidyl rings and not the alkyl-chain region. Consequently, binding should be more specific for nornicotine rather than the nicotine structure bearing a methylated nitrogen.

Cotinine **3** accounts for 70−80% of metabolized nicotine and has a long half-life of 16−20 h.[15] In addition, cotinine levels can average 15-fold higher than nicotine during smoking or nicotine replacement therapy.[15a] Apparently, cotinine also (1) has an effect on nicotinic cholinergic receptors in vitro, (2) influences release of neurotransmitters, (3) inhibits androgen biosynthesis, and (4) possibly contributes to the lower blood pressure of smokers during nonsmoking intervals.[16] Notably, cotinine is reported to effect the pharmacology of nicotine withdrawal and could promote cardiovascular and endocrine effects and withdrawal symptoms after nicotine abstinence.[17] Hence, inclusion of cotinine as a target is warranted in an immunotherapeutic approach to the nicotine problem.

For cotinine-specific antibodies, the (*S*)-hapten **24** (code: **COT**) was synthesized starting from **2b** (Figure 4). The central transformation was the Wenkert oxidation of the amine **21** using conditions similar to those in the

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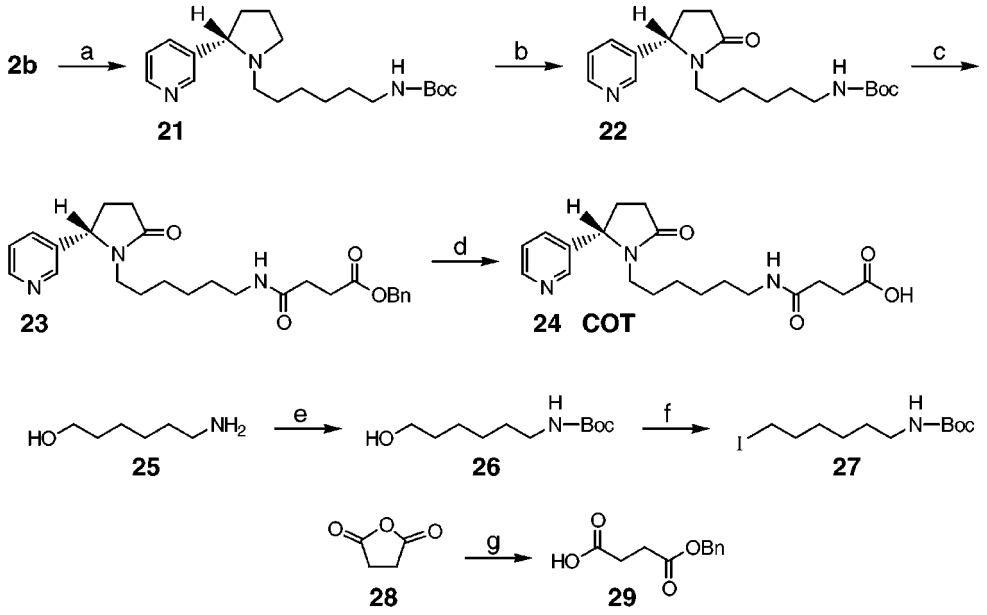

Figure 4. Synthesis of the (*S*)-cotinine hapten: (a) **27**, DIEA, acetonitrile; (b) $Hg(OAc)_2$, aq EDTA, pH 9, dioxane, reflux; (c) (i) TFA, CH_2Cl_2, (ii) **29**, HBTU, *N*-methylmorpholine, DMF; (d) H_2, Pd/C, MeOH; (e) di-*tert*-butyldicarbonate, MeOH; (f) (i) methanesulfonyl chloride, NEt_3, CH_2Cl_2, (ii) NaI, acetonitrile; (g) benzyl alcohol, DMAP, CH_2Cl_2.

literature for the conversion of nicotine to cotinine.[18] The stereochemical integrity should not be affected, since no racemization occurs in this reaction.

Modification of the linker chemistry by using **27** was necessary to provide a compound that was stable during the basic conditions of the oxidation. The benzyl ester-containing linker in **9** decomposed and gave products that could not be purified. Interestingly, unlike in the **NIC** synthesis, the hydrogenation to produce **COT** was nearly quantitative. There was no cleavage of the 5′−C−N bond likely due to deactivation by the pyrrolidone ring and the absence of a basic nitrogen as in **9**. Notably, the final linker in **24** differs from that in **10** by only one bond length while maintaining the desirable internal amide bond.

Coupling of **10** to keyhole limpet hemocyanin (KLH) and bovine serum albumin (BSA) afforded the conjugates **NIC**-KLH for immunization and **NIC**-BSA for screening. Our preliminary studies indicated that **NIC**-KLH could be used to immunize mice in a very consistent fashion and thereby produce both a high quality, reproducible immune response, as well as mAbs with excellent affinity and specificity for nicotine (Table 1). We anticipate similar results for **NOC**-KLH and **COT**-KLH.

The K_d values for nicotine binding surpassed all previously reported examples except those of Langone et al. that were ~10-fold better.[6c] However, the same hapten employed by these workers was used by others and afforded mAbs that bound nicotine ~100-fold less tightly.[6b] Hence, the characteristics of this hapten, or its immunoconjugates, might not be conducive for achieving consistent titers which would be a drawback for therapeutic purposes. Although our antinicotine mAbs had lower affinities, the specificity for nicotine relative to nornicotine and cotinine was improved by severalfold compared to the Langone mAbs which lends support to aspects of our hapten design. Consequently, **2**, **3**, and **4** should not compete with nicotine for antibody binding sites and therefore would not pose a problem during treatment.

Table 1. Data for Some Antinicotine MAbs Derived from NIC−KLH

NIC mAb	isotype (IgG)	K_d (M x 10^7)	specificity[a] (%)
3G2	$\kappa\gamma1$	3.0	0.30, nd
6C12	$\kappa\gamma1$	2.7	0.15, nd
13A3	$\kappa\gamma2a$	1.6	0.10, nd
1B10	$\kappa\gamma1$	2.5	0.20, nd
5E8	$\kappa\gamma1$	3.4	0.65, nd
9D9	$\kappa\gamma1$	2.0	0.055, nd

[a] Determined by enzyme-linked immunosorbent assay (ELISA) using **NIC**-BSA; percent cross-reactivity (**2b**, **3**) relative to the amount of free nicotine that produces 50% decrease in mAb binding; nd = not detectable (no change in ELISA reading at 1000-fold molar excess of **3** compared to nicotine).

While both a nicotine vaccine and an antinicotine mAb are part of our therapeutic strategy, we believe passive immunization protocols would be most practical with regard to **2** and **3**. Certainly, the binding and blockade of nicotine is of paramount importance for therapy. However, the additional treatment with a "cocktail" of mAbs specific for nornicotine and cotinine could have important ramifications with regard to reducing reinforcement and relapse potential, especially for some individuals. The haptens described provide the essential elements in a program of immunopharmacotherapy that offers a new avenue in the challenging battle against nicotine addiction.

Experimental Section

General Methods. ^1H and ^{13}C NMR spectra were measured at 400 MHz on a Brucker AMX-400 spectrometer. Chemical sifts (ppm) were reported relative to internal $CDCl_3$ (^1H, 7.26 ppm and ^{13}C, 77.0 ppm) and CD_3OD (^1H, 3.30 ppm and ^{13}C, 49.2 ppm). HRMS spectra were recorded using electrospray ionization (ESI) or MALDI techniques. Glassware and solvents were dried by standard methods. Flash chromatography was performed on silica gel 60 (230−400 mesh) and thin-layer chromatography on glass plates coated with a 0.02 mm layer

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of silica gel 60 F-254. The *rac*-nornicotine **2a** and other chemical reagents and solvents were from Aldrich Chem. Co., unless otherwise noted, and used without further purification.

Ethyl 5-Bromonicotinate 6. A solution of 5-bromonicotinic acid **5** (20.6 g, 102 mmol) in a mixture of ethanol (400 mL) and concentrated sulfuric acid (5 mL) was stirred at reflux under nitrogen for 18 h. The ethanol was removed and evaporated and the resulting white residue was dissolved in water. The aqueous solution was made basic to pH 8 with sat. sodium bicarbonate and extracted with ether. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated to give **6** as a pale yellow oil (22 g, 94% yield). ^{1}H NMR ($CDCl_3$) δ: 9.08 (d, J = 1.8 Hz, 1H), 8.79 (d, J = 2.4 Hz, 1H), 8.38 (dd, J = 1.8, 2.4 Hz, 1H), 4.38 (q, J = 7.0 Hz, 2H), 1.38 (t, J = 7.0 Hz, 3H). ^{13}C NMR ($CDCl_3$) δ: 163.9, 154.3, 148.7, 139.3, 127.5, 120.5, 61.8, 14.1. HRMS (MALDI−FTMS): calcd for $C_8H_9BrNO_2$ (MH^+) 229.9817, found 229.9816.

3-Bromo-5-(4, 5-dihydro-3*H*-pyrrol-2-yl)-pyridine (5-Bromomyosmine) 7. Sodium hydride (2.4 g, 60 mmol, 60% dispersion in oil) in a three-neck flask was washed with three 20 mL portions of hexane. The flask was fitted with a reflux condenser, flushed with nitrogen, and charged with THF (85 mL). A solution of **6** (10.6 g, 46.1 mmol) and 1-vinyl-2-pyrrolidinone (5.5 g, 49.5 mmol) in THF (15 mL) was added in one portion. The mixture was stirred and refluxed for 1 h and then cooled to room temperature. A solution of concentrated HCl (8 mL) in water (12 mL) was added, and the THF was removed on a rotary evaporator. Additional concentrated HCl (12 mL) and water (24 mL) were added, and the mixture was heated at reflux overnight. In an ice-cooled bath, the solution was made basic with concentrated aqueous NaOH, which resulted in precipitation of the crude product, and then it was extracted with CH_2Cl_2. The CH_2Cl_2 layer was washed with water and brine and evaporated. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/acetone (19:1) to **7** as a pale yellow solid (7.4 g, 72% yield). ^{1}H NMR ($CDCl_3$) δ: 8.82 (d, J = 1.8 Hz, 1H), 8.65 (d, J = 2.4 Hz, 1H), 8.29 (t, J = 2.1 Hz, 1H), 4.06−4.01 (m, 2H), 2.91−2.85 (m, 2H), 2.07−1.98 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 169.7, 152.0, 146.9, 137.0, 131.5, 120.8, 61.6, 34.7, 22.4. MS (ESI): $C_9H_9BrN_2$ (Fw 224/226), m/z 225/227 (MH^+).

3-Bromo-5-(2-pyrrolidinyl)-pyridine (5-Bromonornicotine) 8. Sodium borohydride (4.5 g, 119 mmol) was added portionwise over 10 min with vigorous stirring to a solution of **7** (12.1 g, 53.8 mmol) in 125 mL of 80:20 methanol/acetic acid cooled to −40 °C with a dry ice−acetone bath. During the course of the addition, the temperature rose to −20 °C. After warming to room temperature, most of the solvent was removed with a rotary evaporator. Water (300 mL) was added and the solution was made basic with NaOH and extracted with CH_2Cl_2 (2 × 90 mL). The combined extracts were washed with brine, dried over K_2CO_3, and evaporated. The residue was purified by chromatography on silica gel eluting with EtOAc/MeOH (1:1) to give racemic **8** as a pale yellow oil (9.9 g, 81%). ^{1}H NMR ($CDCl_3$) δ: 8.49 (d, J = 2.1 Hz, 1H), 8.45 (d, J = 1.8 Hz, 1H), 7.87 (t, J = 1.8 Hz, 1H), 4.14 (t, J = 7.6 Hz, 1H), 3.13 (ddd, J = 5.3, 7.6, 10.0 Hz, 1H), 3.02 (ddd, J = 6.8, 7.9, 10.0 Hz, 1H), 2.24−2.16 (m, 1H), 1.93−1.77 (m, 2H), 1.64−1.55 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 149.0, 146.6, 142.7, 136.6, 120.7, 59.1, 46.9, 34.5, 25.4. HRMS (MALDI−FTMS): calcd for $C_9H_{12}BrN_2$ (MH^+) 227.0184, found 227.0175.

Resolution of Racemic 5-Bromonornicotine 8 via the α-Methoxy-α-(trifluoromethyl) Phenylacetate (MTPA) Salts. A solution of (−)-MTPA (3.9 g, 16.7 mmol) in EtOAc (13 mL) was added to a solution of **8** (7.57 g, 33.3 mmol) in EtOAc (52 mL) with stirring. The mixture was allowed to stand at room temperature for 15 min, after which time the crystalline product was collected by filtration to give (*R*)-isomer enriched crystals. Three recrystallizations from boiling acetonitrile yielded 4.1 g (54%) of colorless needles [(*R*)-5-bromonornicotine (−)-MTPA salt]. The filtrate was extracted with 1 N sulfuric acid (2 times). The acid extracts were combined, washed with ether, made basic with NaOH, and extracted with CH_2Cl_2. The organic layer was evaporated and purified on a silica gel column to give an (*S*)-isomer enriched oil. The oil was dissolved in EtOAc (25 mL) and treated with a solution of (+)-MTPA (3.9 g, 16.7 mmol) in EtOAc (13 mL) with stirring. After the solution was left standing for 15 min, the crystallized product was collected by filtration. Three recrystallizations from boiling acetonitrile yielded 4.0 g (52%) of colorless needles [(*S*)-5-bromonornicotine (+)-MTPA salt].

(*S*)-Nornicotine 2b. A suspension of (*S*)-5-bromonornicotine (+)-MTPA salt (2.39 g, 5.17 mmol) in ether (120 mL) was vigorously shaken with 1 M KOH (50 mL) in a separatory funnel. The ether layer was washed with 1 M KOH (50 mL), dried over anhydrous K_2CO_3, and evaporated. The residual oil was dissolved in EtOH (50 mL) containing Et_3N (1.25 mL) and hydrogenated at 1 atm with 10% Pd/C (500 mg). After 1 h, the mixture was filtered through Celite and the filter cake washed with EtOH. The filtrate was poured into 1 M K_2CO_3 (125 mL) which was then extracted with two 125 mL portions of CH_2Cl_2. After washing with brine (50 mL), the combined extracts were dried over anhydrous K_2CO_3 and evaporated. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (7:1) to give (*S*)-**2b** as a pale yellow oil (621 mg, 81% yield). ^{1}H NMR ($CDCl_3$) δ: 8.54 (d, J = 2.1 Hz, 1H), 8.42 (dd, J = 1.8, 5.0 Hz, 1H), 7.67 (dt, J = 1.8, 7.9 Hz, 1H), 7.19 (ddd, J = 0.6, 4.7, 7.9 Hz, 1H), 4.11 (t, J = 7.6 Hz, 1H), 3.15 (ddd, J = 5.6, 7.6, 10.0 Hz, 1H), 3.03−2.97 (m, 1H), 2.21−2.14 (m, 1H), 1.95−1.77 (m, 2H), 1.67−1.57 (m, 1H). ^{13}C NMR ($CDCl_3$) δ: 148.5, 148.1, 140.0, 134.0, 123.2, 59.9, 46.8, 34.2, 25.4. HRMS (MALDI−FTMS): calcd for $C_9H_{13}N_2$ (MH^+) 149.1079, found 149.1074. $[\alpha]^{23}_D$ = −35.8° (c = 2.184, MeOH).

The (*R*)-**2c** isomer was prepared in a similar fashion from (*R*)-5-bromonornicotine (−)-MTPA salt. $[\alpha]^{23}_D$ = + 37.5° (c = 3.806, MeOH).

^{1}H NMR of a diastereomeric derivative was used to determine the enantiomeric excess of each isomer. A small amount of (*S*)-, (*R*)-, or *rac*-nornicotine (∼10 mg) in CH_2Cl_2 (50 μL) was added Et_3N (30 μL, 0.215 mmol) and a solution of (*S*)-α-methoxy-α-(trifluoromethyl)-phenylacetyl chloride (800 μL, 0.1 M in CH_2Cl_2). The reaction mixture was mixed briefly and then evaporated. The residue was partitioned between EtOAc and water, and the EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated. The starting material and byproducts in the residue were removed by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (50:1) to give the amide derivative as a colorless oil (TLC ≥ 90% pure). The 2′-H proton shifted from 4.11 to 5.21 ppm for the (*S*)-nornicotine derivative and to 5.17 ppm for the (*R*)-nornicotine derivative. As indicated by the racemic sample, the patterns were different and clearly distinguishable. Neither diastereomer showed the presence of the other. To obtain a threshold of detection, each isomer was mixed with 1 mol % of the other isomer. Both samples showed a trace signal above noise at the expected location. Hence, it was possible to assign a lower limit of 98% ee to both (*S*)-nornicotine and (*R*)-nornicotine. (*S*)-Nornicotine derivative (2′-H proton) δ: 5.21 (dd, J = 3.8, 6.2 Hz, 1H). HRMS (MALDI−FTMS): calcd for $C_{19}H_{20}F_3N_2O_2$ (MH^+) 365.1471, found 365.1474. (*R*)-Nornicotine derivative (2′-H proton) δ: 5.17 (dd, J = 6.8, 7.9 Hz, 1H). HRMS (MALDI−FTMS): calcd for $C_{19}H_{20}F_3N_2O_2$ (MH^+) 365.1471, found 365.1483.

***N*-[1-Oxo-6-[(2*S*)-2-(3-pyridinyl)-1-pyrrolidinyl]hexyl]-*β*-alanine Phenylmethyl Ester 9.** A solution of **14** (136 mg, 0.338 mmol) in acetonitrile (500 μL) was added to a mixture of **2b** (52.1 mg, 0.338 mmol) and diisopropylethylamine (DIEA) (117 μL, 0.676 mmol) in acetonitrile (850 μL) with stirring at room temperature. After 18 h, the mixture was evaporated and the residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (19:1) to give product **9** as a pale yellow oil (72 mg, 48% yield). ^{1}H NMR ($CDCl_3$) δ: 8.53 (br s, 1H), 8.46 (br d, 1H), 7.67 (br d, 1H), 7.36−7.31 (m, 5H), 7.23 (dd, J = 4.7, 7.9 Hz, 1H), 6.07 (br s, 1H), 5.12 (s, 2H), 3.50 (q, J = 5.9, 2H), 3.31 (dt, J = 2.1, 8.2 Hz, 1H), 3.23 (t, J = 8.2 Hz, 1H), 2.57 (t, J = 5.9 Hz, 2H), 2.43 (dt, J = 8.2, 11.7 Hz, 1H), 2.20−2.13 (m, 2H), 2.05 (t, J = 8.2 Hz, 2H), 2.07−2.01 (m, 1H), 1.97−1.85 (m, 1H), 1.85−1.76 (m, 1H), 1.69−1.60 (m, 1H), 1.53−1.45 (m, 2H), 1.43−1.36 (m, 2H), 1.32−1.13 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.9, 172.5, 149.4, 148.4, 135.6, 134.9, 128.6,

128.4, 128.2, 123.5, 67.6, 66.5, 54.0, 53.5, 36.6, 35.1, 34.7, 34.1, 28.4, 26.9, 25.4, 22.6. HRMS (MALDI−FTMS): calcd for $C_{25}H_{34}N_3O_3$ (MH^+) 424.2595, found 424.2607. $[\alpha]^{23}_D = -65.3°$ (c = 1.432, MeOH).

***N*-[1-Oxo-6-[(2*S*)-2-(3-pyridinyl)-1-pyrrolidinyl]hexyl]-*β*-alanine 10 (NIC).** The benzylester **9** (51.4 mg, 0.122 mmol) in MeOH (2 mL) was hydrogenated with 10% Pd/C (9.3 mg) at 40 psi. After 90 min, the mixture was filtered through Celite and the filter cake was washed with MeOH. The filtrate was evaporated and the residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (3:2) to give hapten **10** as a pale yellow oil (26 mg, 66% yield). ^{1}H NMR (CD_3OD) δ: 8.53 (s, 1H), 8.45 (d, J = 3.5 Hz 1H), 7.93−7.90 (m, 1H), 7.44 (dd, J = 5.0, 7.9 Hz, 1H), 3.55 (t, J = 8.8 Hz, 1H), 3.49−3.43 (m, 1H), 3.38 (t, J = 6.8 Hz, 2H), 2.58−2.51 (m, 1H), 2.50−2.43 (m, 1H), 2.37 (t, J = 6.8 Hz, 2H), 2.32−2.24 (m, 2H), 2.11 (t, J = 7.3 Hz, 2H), 2.07−1.90 (m, 2H), 1.86−1.76 (m, 1H), 1.55−1.45 (m, 4H), 1.34−1.17 (m, 2H). ^{13}C NMR (CD_3OD) δ: 179.1, 176.0, 150.2, 149.7, 139.3, 138.0, 125.7, 69.5, 55.4, 54.8, 37.6, 37.6, 37.1, 35.2, 28.8, 28.0, 26.8, 23.5. HRMS (MALDI−FTMS): calcd for $C_{18}H_{28}N_3O_3$ (MH^+) 334.2131, found 334.2139. $[\alpha]^{23}_D = -71.0°$ (c = 0.534, MeOH).

***N*-(1-Oxo-6-bromohexyl)-*β*-alanine 13.** A mixture of 6-bromohexanoic acid **11** (2.20 g, 11.4 mmol) and HBTU (International Peptides) (12.5 mmol) in DMF (15 mL) was stirred at 0 °C for 10 min. To the reaction mixture was added a solution of β-alanine benzyl ester *p*-TsOH salt **12** (Sigma) (4.00 g, 11.4 mmol) and *N*-methyl morpholine (3.75 mL) in DMF (10 mL) at 0 °C with stirring. After 24 h at room temperature, the reaction mixture was poured into water and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane/EtOAc (1:1) to **13** as a pale yellow oil (3.4 g, 84% yield). ^{1}H NMR ($CDCl_3$) δ: 7.37−7.34 (m, 5H), 6.02 (br s, 1H), 5.14 (s, 2H), 3.52 (q, J = 5.9 Hz, 2H), 3.39 (t, J = 6.8 Hz, 2H), 2.59 (t, J = 5.9 Hz, 2H), 2.13 (t, J = 7.6 Hz, 2H), 1.89−1.82 (m, 2H), 1.67−1.58 (m, 2H), 1.47−1.40 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.6, 172.4, 135.5, 128.5, 128.3, 128.2, 66.5, 36.2, 34.7, 34.0, 33.6, 32.3, 27.6, 24.6. HRMS (MALDI−FTMS): calcd for $C_{16}H_{22}BrNO_3Na$ (MNa^+) 378.0681, found 378.0693.

***N*-(1-Oxo-6-iodohexyl)-*β*-alanine 14.** A mixture of **13** (1.00 g, 2.81 mmol) and sodium iodide (2.10 g, 14.1 mmol) in acetonitrile (6 mL) was vigorously stirred at room temperature for 18 h. The progress of the reaction was followed by ^{1}H NMR. After completion, the mixture was evaporated and the residue was partitioned between EtOAc and water. The EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane/EtOAc (1:1) to give **14** as a colorless oil (1.11 g, 98% yield). ^{1}H NMR ($CDCl_3$) δ: 7.38−7.32 (m, 5H), 6.08 (br s, 1H), 5.13 (s, 2H), 3.51 (q, J = 6.2 Hz, 2H), 3.16 (t, J = 7.0 Hz, 2H), 2.58 (t, J = 6.2 Hz, 2H), 2.12 (t, J = 7.6 Hz, 2H), 1.84−1.76 (m, 2H), 1.64−1.56 (m, 2H), 1.42−1.34 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.6, 172.5, 135.6, 128.6, 128.4, 128.2, 66.5, 36.2, 34.7, 34.0, 33.0, 29.9, 24.4, 6.7. HRMS (MALDI−FTMS): calcd for $C_{16}H_{22}INO_3Na$ (MNa^+) 426.0537, found 426.0553.

***rac*-2-(3-Pyridinyl)-1-pyrrolidinebutanoic Acid Phenylmethyl Ester 16a.** A solution of **20** (103 mg, 0.338 mmol) in acetonitrile (500 μL) was added to a solution of *rac*-nornicotine (50.0 mg, 0.338 mmol) and DIEA (117 μL, 0.676 mmol) in CH_3CN (850 μL) with stirring at room temperature. After 18 h, the reaction mixture was evaporated and the residue was purified by chromatography on silica gel, eluting with CH_2Cl_2/MeOH (19:1) to give **16a** as a pale yellow oil (65 mg, 59% yield). ^{1}H NMR (CD_3OD) δ: 8.46 (br s, 1H), 8.38 (d, J = 4.7 Hz, 1H), 7.82−7.79 (m, 1H), 7.35−7.29 (m, 6H), 5.06 (d, J = 12.3 Hz, 1H), 5.00 (d, J = 12.3 Hz, 1H), 3.36−3.28 (m, 2H), 2.44 (dt, J = 8.2, 12.0 Hz, 1H), 2.32 (dt, J = 2.1, 7.0 Hz, 2H), 2.24−2.11 (m, 3H), 1.96−1.81 (m, 2H), 1.80−1.69 (m, 2H), 1.67−1.57 (m, 1H). ^{13}C NMR (CD_3OD) δ: 174.9, 149.9, 149.0, 141.7, 137.8, 137.6, 129.7, 129.4, 129.4, 125.5, 68.9, 67.3, 54.7, 54.5, 36.2, 32.9, 25.0, 23.7. HRMS (MALDI−FTMS): calcd for $C_{20}H_{25}N_2O_2$ (MH^+) 325.1911, found 325.1900.

(2*S*)-2-(3-Pyridinyl)-1-pyrrolidinebutanoic Acid Phenylmethyl Ester 16b. Linker **20** (198 mg, 0.650 mmol), (*S*)-nornicotine **2b** (96.3 mg, 0.650 mmol), DIEA (226 μL, 1.30 mmol). Pale yellow oil (124 mg, 58% yield). $[\alpha]^{23}_D = -88.7°$ (c = 1.076, MeOH).

(2*R*)-2-(3-Pyridinyl)-1-pyrrolidinebutanoic Acid Phenylmethyl Ester 16c. Linker **20** (198 mg, 0.650 mmol), (*R*)-nornicotine **2c** (96.3 mg, 0.650 mmol), DIEA (226 μL, 1.30 mmol). Pale yellow oil (131 mg, 62% yield). $[\alpha]^{23}_D = +87.5°$ (c = 1.072, MeOH).

***rac*-2-(3-Pyridinyl)-1-pyrrolidinebutanoic Acid 17a (*rac*-NOC).** The benzylester **16a** (58.3 mg, 0.180 mmol) in MeOH (1.5 mL) was hydrogenated with 10% Pd/C (15 mg) using a balloon technique. After 75 min, the reaction mixture was filtered through Celite and the filter cake was washed with MeOH. The filtrate was evaporated, and the residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (3:2) to give **17a** as a colorless oil (26 mg, 62% yield). ^{1}H NMR (CD_3OD) δ: 8.60 (d, J = 1.8 Hz, 1H), 8.53 (dd, J = 1.8, 5.0 Hz, 1H), 7.98 (dt, J = 2.1, 7.9 Hz, 1H), 7.48 (ddd, J = 0.9, 5.0, 7.9 Hz, 1H), 3.94 (dd, J = 7.6, 9.4 Hz, 1H), 3.71−3.65 (m, 1H), 2.85−2.69 (m, 3H), 2.43−2.38 (m, 1H), 2.33−2.26 (m, 1H), 2.17−1.98 (m, 4H), 1.83−1.71 (m, 2H). ^{13}C NMR (CD_3OD) δ: 179.7, 150.7, 150.6, 138.0, 136.1, 125.9, 69.5, 55.3, 54.6, 35.8, 34.4, 24.0, 23.3. HRMS (MALDI−FTMS): calcd for $C_{13}H_{19}N_2O_2$ (MH^+) 235.1441, found 235.1440.

(2*S*)-2-(3-Pyridinyl)-1-pyrrolidinebutanoic Acid 17b [(*S*)-NOC]. The benzylester **16b** (120 mg, 0.370 mmol), 10% Pd/C (30 mg), reaction time 20 min. Colorless oil (63 mg, 73% yield). $[\alpha]^{23}_D = -15.7°$ (c = 1.202, MeOH).

(2*R*)-2-(3-Pyridinyl)-1-pyrrolidinebutanoic Acid 17c [(*R*)-NOC]. The benzylester **16c** (95.0 mg, 0.293 mmol), 10% Pd/C (25 mg), reaction time 20 min. Colorless oil (41 mg, 59% yield). $[\alpha]^{23}_D = +13°$ (c = 0.822, MeOH).

Benzyl 4-Bromobutanoate 19. A solution of 4-bromobutanoic acid **18** (25 g, 0.15 mol), benzyl alcohol (21 g, 0.194 mol), and *p*-TsOH hydrate (1.3 g, 6.87 mmol) in cyclohexane (225 mL) was heated to reflux. The water was azeotropically removed with the aid of a Dean−Stark trap. After 1 hr, the stoichiometric amount of water was collected and the solution refluxed an additional hr. After cooling to room temperature, the solution was washed with sat. sodium bicarbonate and brine and dried over Na_2SO_4 and evaporated. The residue was distilled (bp 120−125 °C, 2 mm Hg) to give **19** as a clear, colorless liquid (30 g, 78% yield).^{1}H NMR ($CDCl_3$) δ: 7.40−7.32 (m, 5H), 5.13 (s, 2H), 3.46 (t, J = 6.5 Hz, 2H), 2.56 (t, J = 7.1 Hz, 2H), 2.23−2.16 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.3, 135.7, 128.5, 128.3, 128.2, 66.4, 32.7, 32.4, 27.7.

Benzyl 4-Iodobutanoate 20. A mixture of **19** (500 mg, 1.95 mmol) and sodium iodide (1.46 g, 9.75 mmol) in acetonitrile (4 mL) was vigorously stirred at room temperature for 18 h. The progress of the reaction was followed by ^{1}H NMR. After completion, the reaction mixture was evaporated and the residue was partitioned between EtOAc and water. The EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane/CH_2Cl_2 (2:1) to give **20** as a colorless oil (466 mg, 79% yield). ^{1}H NMR ($CDCl_3$) δ: 7.39−7.34 (m, 5H), 5.14 (s, 2H), 3.23 (t, J = 6.8 Hz, 2H), 2.51 (t, J = 7.1 Hz, 2H), 2.19−2.12 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 172.0, 135.7, 128.5, 128.2, 128.1, 66.3, 34.7, 28.3, 5.4. MS (GCMS) $C_{11}H_{13}IO_2$ (Fw 304), m/z 304 (M^+).

[6-[(2*S*)-2-(3-Pyridinyl)-1-pyrrolidinyl]hexyl]-carbamic Acid 1,1-Dimethylethyl Ester 21. A solution of **27** (425 mg, 1.30 mmol) in acetonitrile (2 mL) was added to a mixture of **2b** (200 mg, 1.35 mmol) and DIEA (470 μL, 2.70 mmol) in acetonitrile (4 mL) with stirring at room temperature. After 15 h, the reaction mixture was evaporated and the residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (30:1) to give **21** as a pale yellow oil (266 mg, 59% yield). ^{1}H NMR (CD_3OD) δ: 8.49 (s, 1H), 8.41 (d, J = 3.8 Hz, 1H), 7.85 (dt, J = 1.8, 7.9 Hz, 1H), 7.40 (dd, J = 5.0, 7.9 Hz, 1H), 3.36−3.29 (m, 2H), 2.96 (t, J = 7.0 Hz, 2H), 2.44 (dt, J = 8.5, 12.0 Hz, 1H), 2.29−2.07 (m, 2H), 2.13 (m, 1H), 1.96−1.85 (m, 2H), 1.72−1.63 (m, 1H), 1.46−1.35 (m, 13H), 1.30−1.18

(m, 4H). ^{13}C NMR (CD_3OD) δ: 158.7, 149.9, 149.0, 141.7, 137.7, 125.5, 79.9, 69.2, 55.7, 54.9, 41.4, 36.0, 31.1, 29.8, 29.0, 28.4, 27.9, 23.6. HRMS (MALDI−FTMS): calcd for $C_{20}H_{34}N_3O_2$ (MH^+) 348.2646, found 348.2630. $[\alpha]^{23}_D = -74.2°$ ($c = 0.96$, MeOH).

[6-[(5*S*)-2-Oxo-5-(3-pyridinyl)-1-pyrrolidinyl]hexyl]-carbamic Acid 1,1-Dimethylethyl Ester 22. A solution of **21** (265 mg, 0.764 mmol) in dioxane (30 mL) was added to a mixture of $Hg(OAc)_2$ (1.22 g, 3.82 mmol) and aqueous EDTA (7.66 mL, 5 mmol/10 mL, pH 9.0) in water (39 mL), and the mixture was refluxed with stirring for 2 h. After cooling, the mixture was filtered through Celite and the filter cake was washed with a small amount of dioxane. The filtrate was extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (25:1) to give **22** as a pale yellow oil (95 mg, 34% yield). ^{1}H NMR (CD_3OD) δ: 8.52 (dd, $J = 1.5$, 5.0 Hz, 1H), 8.50 (d, $J = 1.5$ Hz, 1H), 7.78 (dt, $J = 2.1$, 7.9 Hz, 1H), 7.49 (ddd, $J = 0.9$, 5.0, 7.9 Hz, 1H), 6.54 (br s, 1H), 4.88−4.85 (m, 1H), 3.61−3.53 (m, 1H), 3.00−2.95 (m, 2H), 2.64−2.48 (m, 4H), 1.95−1.89 (m, 1H), 1.42−1.35 (m, 13H), 1.28−1.19 (m, 4H). ^{13}C NMR (CD_3OD) δ: 178.2, 158.7, 150.3, 149.4, 139.0, 136.9, 126.0, 79.9, 61.5, 42.1, 41.4, 31.3, 30.9, 29.3, 29.0, 27.9, 27.6, 27.5. HRMS (MALDI−FTMS): calcd for $C_{20}H_{31}N_3O_3Na$ (MNa^+) 384.2258, found 384.2273. $[\alpha]^{23}_D = -16.4°$ ($c = 1.834$, MeOH).

4-Oxo-4-[[6-[(5*S*)-2-oxo-5-(3-pyridinyl)-1-pyrrolidinyl]]-hexyl]amino]-butanoic Acid Phenylmethyl Ester 23. A solution of **22** (91.7 mg, 0.254 mmol) in CH_2Cl_2 (500 μL) was treated with TFA (400 μL) at 0 °C. After completion, the mixture was evaporated with toluene (3 times). HBTU (111 mg, 0.292 mmol) was added to a solution of **29** (58.1 mg, 0.279 mmol) in DMF (500 μL) at 0 °C with stirring. After 10 min, a solution of the deprotected amine residue and *N*-methylmorpholine (167 μL, 1.52 mmol) in DMF (300 μL) was added at 0 °C with stirring. After 18 h at room temperature, the mixture was partitioned between EtOAc and water. The EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (35:1) to give **23** as a pale yellow oil (79 mg, 69% yield). ^{1}H NMR (CD_3OD) δ: 8.52 (dd, $J = 1.5$, 4.7 Hz, 1H), 8.49 (d, $J = 1.5$ Hz, 1H), 7.77 (dt, $J = 1.8$, 7.9 Hz, 1H), 7.48 (dd, $J = 4.7$, 7.9, Hz, 1H), 7.34−7.28 (m, 5H), 5.10 (s, 2H), 4.86−4.83 (m, 1H), 3.56 (dt, $J = 7.6$, 13.8 Hz, 1H), 3.09 (dt, $J = 2.1$, 7.0 Hz, 2H), 2.65 (t, $J = 6.8$ Hz, 2H), 2.63−2.51 (m, 4H), 2.47 (t, $J = 6.8$ Hz, 2H), 1.96−1.88 (m, 1H), 1.42−1.35 (m, 4H), 1.27−1.16 (m, 4H). ^{13}C NMR (CD_3OD) δ: 178.1, 174.2, 150.3, 149.4, 139.0, 137.8, 136.9, 129.7, 129.3, 129.3, 126.0, 67.5, 61.4, 42.0, 40.3, 31.6, 31.3, 30.7, 30.3, 29.2, 27.9, 27.5. HRMS (MALDI−FTMS): calcd for $C_{26}H_{33}N_3O_4Na$ (MNa^+) 474.2363, found 474.2384. $[\alpha]^{23}_D = -12.6°$ ($c = 1.598$, MeOH).

4-Oxo-4-[[6-[(5*S*)-2-oxo-5-(3-pyridinyl)-1-pyrrolidinyl]]-hexyl]amino]-butanoic Acid 24 (COT). The benzyl ester **23** (77.0 mg, 0.171 mmol) in MeOH (2 mL) was hydrogenated with 10% Pd/C (15 mg) using a balloon technique. After 40 min, Pd/C was removed by filtration washing with MeOH. The filtrate was evaporated, and the residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (3:1) to give **24** as a pale yellow oil (59 mg, 96% yield). ^{1}H NMR (CD_3OD) δ: 8.51 (br s, 2H), 7.79 (dt, $J = 1.8$, 7.9 Hz, 1H), 7.49 (dd, $J = 5.0$, 7.9 Hz, 1H), 4.88−4.85 (m, 1H), 3.57 (dt, $J = 7.6$, 14.1 Hz, 1H), 3.11 (dt, $J = 1.2$, 7.0 Hz, 2H), 2.63−2.4 (m, 8H), 1.97−1.89 (m, 1H), 1.46−1.35 (m, 4H), 1.30−1.17 (m, 4H). ^{13}C NMR (CD_3OD) δ: 179.7, 178.1, 175.5, 150.3, 149.4, 139.0, 136.9, 126.0, 61.4, 42.0, 40.4, 33.0, 31.3, 30.3, 29.2, 27.9, 27.5. HRMS (MALDI−FTMS): calcd for $C_{19}H_{27}N_3O_4Na$ (MNa^+) 384.1894, found 384.1898. $[\alpha]^{23}_D = -14.2°$ ($c = 1.186$, MeOH).

(6-Hydroxyhexyl)-carbamic Acid 1,1-Dimethylethyl Ester 26. A mixture of 6-aminohexanol **25** (2.0 g 17.1 mmol) and di-*tert*-butyldicarbonate (4.1 g, 18.8 mmol) in MeOH (40 mL) was stirred at room temperature for 90 min. The reaction mixture was evaporated, and the residue was purified by chromatography on silica gel eluting with CH_2Cl_2/MeOH (49:1) to give **26** as a pale yellow oil (3.7 g, 99%). ^{1}H NMR ($CDCl_3$) δ: 4.63 (br s, 1H), 3.58 (t, $J = 6.5$ Hz, 2H), 3.07 (q, $J = 6.5$ Hz, 2H), 2,12 (br s, 1H), 1.56−1.49 (m, 2H), 1.48−1.40 (m, 11H), 1.37−1.27 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 156.0, 79.0, 62.4, 40.3, 32.5, 29.9, 28.3, 26.3, 25.2. MS (ESI): $C_{11}H_{23}NO_3$ (Fw 217), m/z 240 (MNa^+).

(6-Iodohexyl)-carbamic Acid 1,1-Dimethylethyl Ester 27. To a mixture of **26** (1.55 g, 7.13 mmol) and Et_3N (1.5 mL, 10.7 mmol) in CH_2Cl_2 (20 mL) in an ice-cooled bath was added mesyl chloride (607 μL, 7.8 mmol) with stirring. After 2 h at room temperature, the reaction mixture was washed with water and brine, dried over Na_2SO_4, and evaporated. The residue was dissolved in acetonitrile (30 mL), and then sodium iodide (5.35 g, 35.7 mmol) was added at room temperature with stirring. After 18 h, the mixture was evaporated and the residue was partitioned between EtOAc and water. The organic layer was washed with brine, dried over Na_2SO_4, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane/EtOAc (10:1) and to give **27** as a colorless oil (1.8 g, 77% yield). ^{1}H NMR ($CDCl_3$) δ: 4.55 (br s, 1H), 3.16 (t, $J = 6.8$ Hz, 2H), 3.08 (q, $J = 6.5$ Hz, 2H) 1.80 (m, 2H), 1.48−1.35 (m, 13H), 1.34−1.28 (m, 2H). ^{13}C NMR ($CDCl_3$) δ: 155.9, 79.0, 40.4, 33.3, 30.1, 29.8, 28.3, 25.6, 6.9. MS (ESI): $C_{11}H_{22}INO_2$ (Fw 327), m/z 350 (MNa^+).

Succinic Acid Monobenzyl Ester 29. A mixture of succinic anhydride **28** (1.00 g, 10.0 mmol), benzyl alcohol (860 μL, 8.30 mmol), and DMAP (1.02 g, 8.30 mmol) in CH_2Cl_2 (30 mL) was stirred at room temperature for 18 h. After this time, a solution of 5% Na_2CO_3 was poured into the reaction mixture and the layers separated. The aqueous layer was acidified with 1 M HCl and extracted with EtOAc. The EtOAc layer was washed with brine, dried over Na_2SO_4, and evaporated to give **29** as a colorless oil (1.63 g, 94%). ^{1}H NMR ($CDCl_3$) δ: 7.37−7.34 (m, 5H), 5.15 (s, 2H), 2.74−2.66 (m, 4H). ^{13}C NMR ($CDCl_3$) δ: 178.4, 172.0, 135.6, 128.5, 128.2, 128.1, 66.6, 28.9, 28.8. HRMS (MALDI−FTMS): calcd for $C_{11}H_{12}O_4Na$ (MNa^+) 231.0628, found 231.0627.

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