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Directed Molecular Recognition: Design and Synthesis of Neutral Receptors for Biotin To Bind Both Its Functional Groups

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Received May 17, 2006

The neutral receptors **1** and **2** are designed and synthesized for the recognition of biotin, a biologically significant molecule, in chloroform to bind completely both of its functional groups simultaneously, i.e., cyclic urea and the carboxyl groups. The truncated receptor **3** binds only the cyclic urea moiety.

Introduction

The molecular recognition implies a pattern of recognition process through a structurally well-defined set of intermolecular interactions between the host and the guest substrates.[1] Multiple hydrogen bonds have a good logical path to achieve and direct the molecular recognition process. The specificity in molecular recognition is related to the strength and number of hydrogen bonds.[2] In this connection, we have already reported[3] the recognition of mono- and dicarboxylic acids,[3a,b] hydroxy acid (tartaric acid),[3b] and amino acid,[3c] and also inhibition of hydrogen bonding in molecular recognition.[4] The directed molecular recognition by design, synthesis, and binding studies of specific receptors for various types of biologically important substrates, e.g. binuclear substrates such as adenine,[5a] caffeine,[5b] uric acid, and also small molecules such as urea,[5c,d] creatinine, etc. has been our keen interest[5] where we have effectively complexed by hydrogen bonding and/or π-stacking forces as demonstrated by the solubilization of these polar guest substrates in less polar solvents such as chloroform. Biotin is an essential vitamin (anti-egg white injury factor) that functions as an indispensable co-enzyme in the mammalian metabolism in a range of biocarboxylations related to crucial physiological processes such as gluconeogenesis and fatty acid biosynthesis.[6]

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10.1021/jo061013j CCC: $33.50
Published on Web 08/17/2006

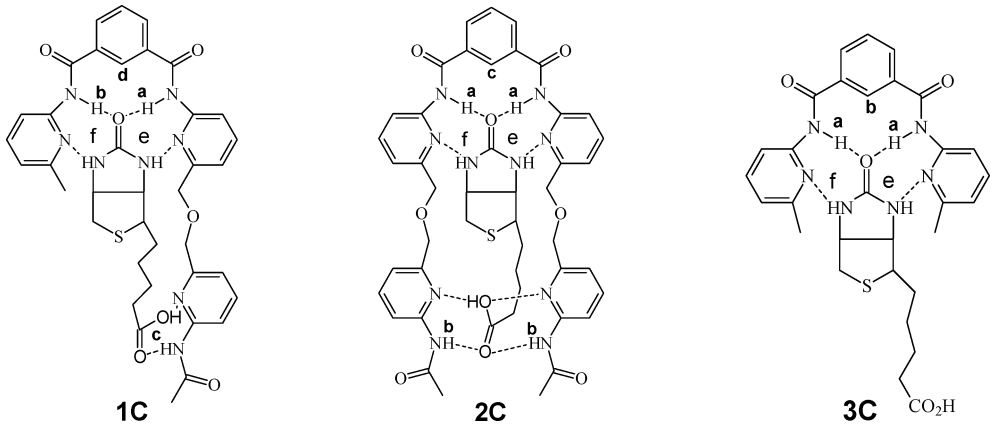
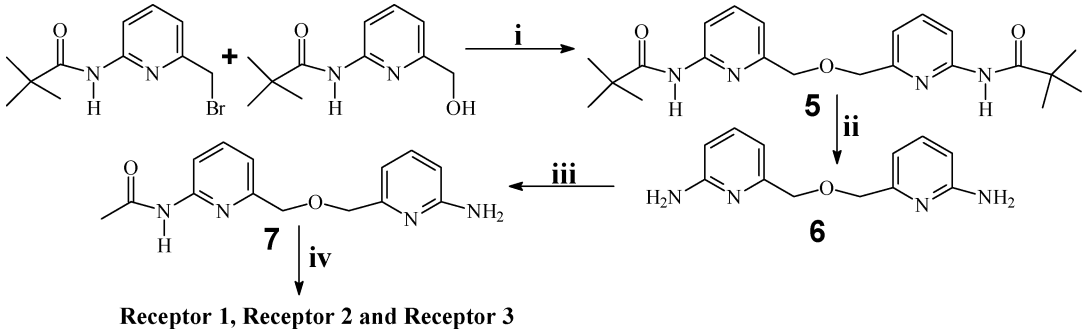


FIGURE 1. Possible modes of complexation: **1C** (receptor **1** with biotin); **2C** (receptor **2** with biotin), and **3C** (truncated receptor **3** with biotin).

SCHEME 1. Synthesis of Receptors 1, 2, and 3[a]



[a] Reagents and conditions: (i) NaH, dry THF, 12 h, rt, 68%; (ii) 4 N KOH−EtOH (1:1); reflux, 6 h, 95%; (iii) acetic anhydride, dry CH_2Cl_2, 2 h, rt, 42%; (iv) 2-amino-6-methylpyridine, isophthaloyl chloride, Et_3N, dry CH_2Cl_2, 24 h, rt (isolated yield: 17% for receptor **1**, 22% for receptor **2** and 26% for receptor **3**).

Several elegant total syntheses of this biologically important molecule have been reported.[7] Biotin contains a cyclic urea linkage with a fused tetrahydrothiophene nucleus where both the hydrogens at the ring junctions and the hydrogen attached to the asymmetric center bearing the *n*-petanoic acid side chain to the tetrahydrothiophene nucleus are all *syn*. The presence of two such polar groups makes biotin notoriously insoluble in chloroform. Thus it is an interesting and critical problem in the molecular recognition study of biotin to bind both the polar groups and thereby solubilize biotin itself in less polar organic solvents. The crystal structure of biotin[8] shows the carboxyl group of one biotin molecule is intermolecularly hydrogen bonded to the urea linkage of the other biotin molecule. Receptors **1** and **2** are designed to bind both the functional groups (urea and carboxyl moieties) of biotin by breaking these self-intermolecular hydrogen bonds in the guest biotin itself to form hetero-hydrogen bonds with the host receptors. In contrast to amino acid, the carboxylic acid and urea moieties in biotin do not exist as zwitterions because urea linkage is not as basic as amino group. Wilcox and co-workers[9] reported an elegant Troger's base receptor having two carboxyl groups that bind biotin methyl ester (soluble in chloroform unlike biotin itself) where the cyclic urea part in the biotin ester was only available for binding and not the carboxyl group present in biotin. Recently Claramunt et al. have studied the recognition of biotin methyl ester using our previously reported receptor (**3**) along with a new receptor.[10] But there is no report yet in the literature to our knowledge of a complete receptor for biotin itself where both its important functional groups, the top urea part and the carboxyl moiety, are involved in binding and it is still a critical unsolved problem.

This paper describes the first model of tandem binding of biotin. The present approach is to design neutral complete receptors **1** and **2** that direct their own functional groups for complexation with both moieties of the guest biotin compared to the truncated partial diamide receptor **3**, which binds only with the urea part of biotin. The neutral triamide receptors (**1** and **2**) are soluble in common organic solvents such as chloroform, dichloromethane, etc. and we have attempted to solubilize biotin via complexation with the receptors in chloroform, which is a common NMR solvent having comparable polarity to that of the interior cavity of an enzyme.

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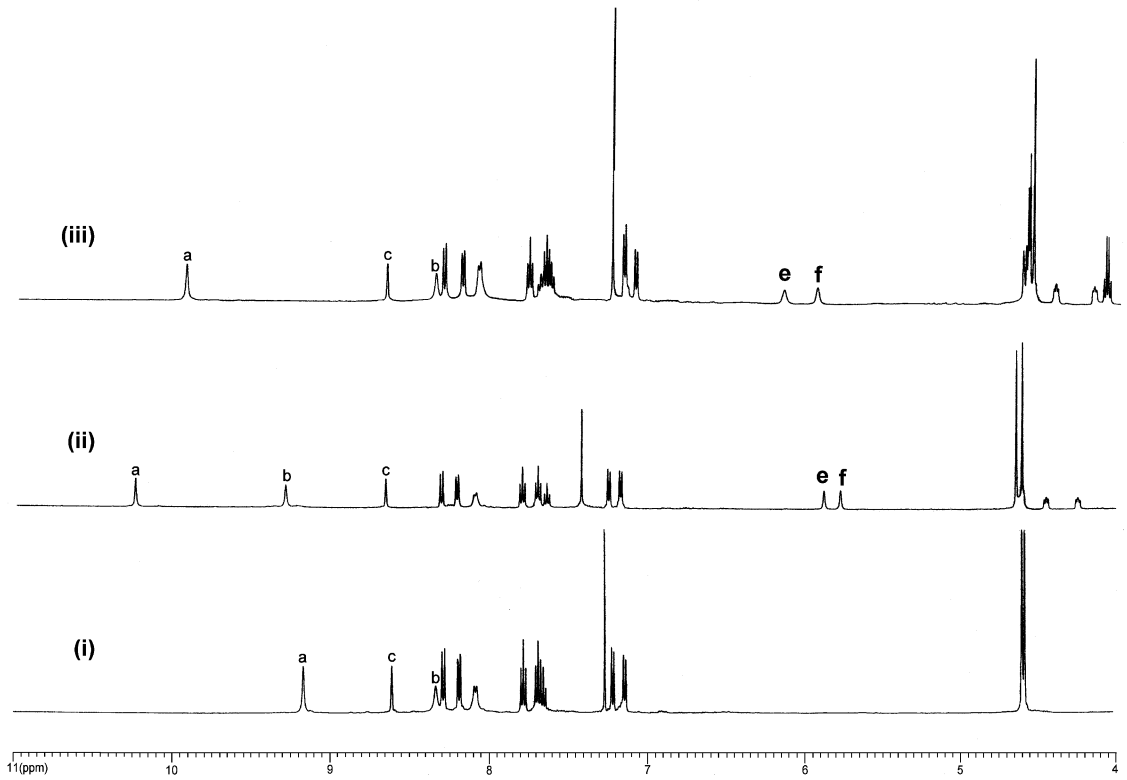


FIGURE 2. ^{1}H NMR spectra of (i) receptor **1** in $CDCl_3$, (ii) receptor **1** with biotin in 2% d_6-DMSO in $CDCl_3$, and (iii) receptor **1** with ethyl ester of biotin in $CDCl_3$ (for a, b, c, d, e, and f refer to Figure 1).

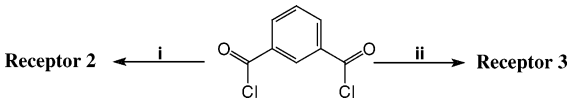
The flexible cavity in the open but semirigid receptors **1** and **2** is specially designed based on the fact that their isophthaloyl pyridine diamide moieties are both directed downward for the complementary hydrogen bonding with the urea part[5] (as also shown by the truncated receptor **3**) and the terminal pyridine amide of the receptors **1** and **2** are free to bind the carboxyl moiety of the guest biotin. The possible modes of complexation are thus shown in **1C**, **2C**, and **3C**, respectively (Figure 1). Hydrogen bonding points are thus complimentarily well arranged in the complexes of receptors **1** and **2**. All the donor and acceptor hydrogen bonding interactions between these receptors and biotin can then be saturated.

Results and Discussion

The combinatorial approach for the synthesis of all the desired receptors **1**, **2**, and **3** has been successfully carried out by the one-step high-dilution reaction followed by preparative chromatography (Scheme 1). 2-(*N*-Pivaloylamino)-6-hydroxymethylpyridine and 2-(*N*-pivaloylamino)-6-hydroxymethylpyridine are coupled in the presence of sodium hydride in dry THF at room temperature to prepare compound **5**.[11] Compound **5** on hydrolysis under reflux with 4 N KOH ethanol−water (1:1) solution produces **6**. Then **7** is obtained from **6** by controlled acetylation with acetic anhydride in dichloromethane (2 h of stirring). Finally receptors **1**, **2**, and **3** are isolated from the reactions of **7**, 2-amino-6-methylpyridine, and isophthaloyl chloride by high dilution technique.

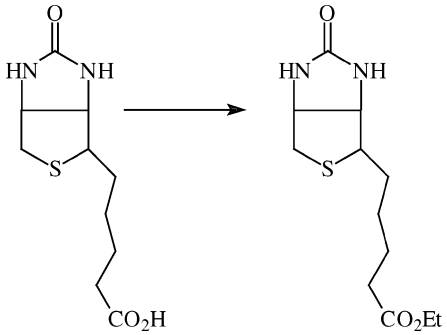
The coupling of isophthaloyl chloride and compound **7** also produced the receptor **2**. Similarly the receptor **3** was simply synthesized by coupling isophthaloyl chloride with 2-aminopicoline (Scheme 2). From the NMR studies, large δ shifts of the key binding protons are observed when all the receptors undergo 1:1 complexation respectively with biotin in 2% d_6-DMSO in $CDCl_3$. The two pyridine amide protons which are flanked by isophthaloyl spacer in unsymmetrical receptor **1** do not differentiate and appear at the same position (δ 8.94 ppm). But on complexation with the urea linkage of biotin, these are shifted and distinctly differentiated. The formation of a tight complex

SCHEME 2. Synthesis of Receptors 2 and 3[a]



[a] Reagents and conditions: (i) compound **7**, Et_3N, dry CH_2Cl_2, 24 h, rt, 58%; (ii) 2-amino-6-methylpyridine, Et_3N, dry CH_2Cl_2, 24 h, rt, 72%.

SCHEME 3. Synthesis of Compound 4 (biotin ethyl ester)[a]



[a] Reagents and conditions: (i) biotin, ethanol, two drops concentrated H_2SO_4, 24 h, reflux, 90%.

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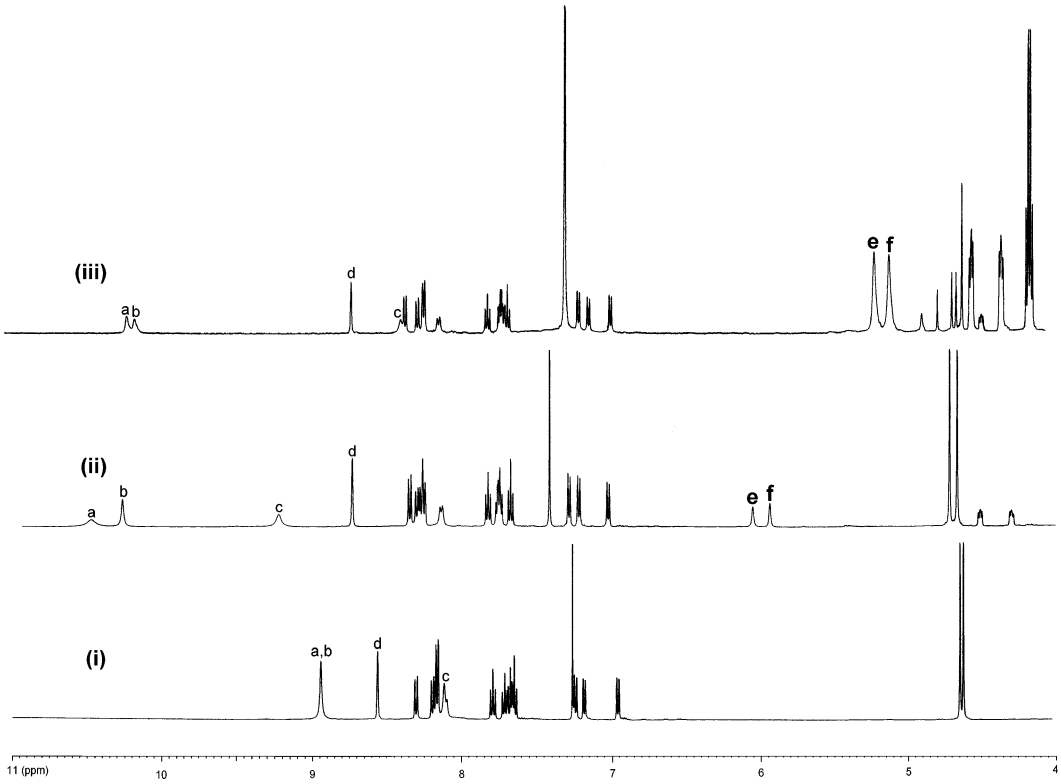

FIGURE 3. ^{1}H NMR spectra of (i) receptor **2** in $CDCl_3$, (ii) receptor **2** with biotin in 2% d_6-DMSO in $CDCl_3$, and (iii) receptor **2** with ethyl ester of biotin in $CDCl_3$ (for a, b, c, e, and f refer to Figure 1).

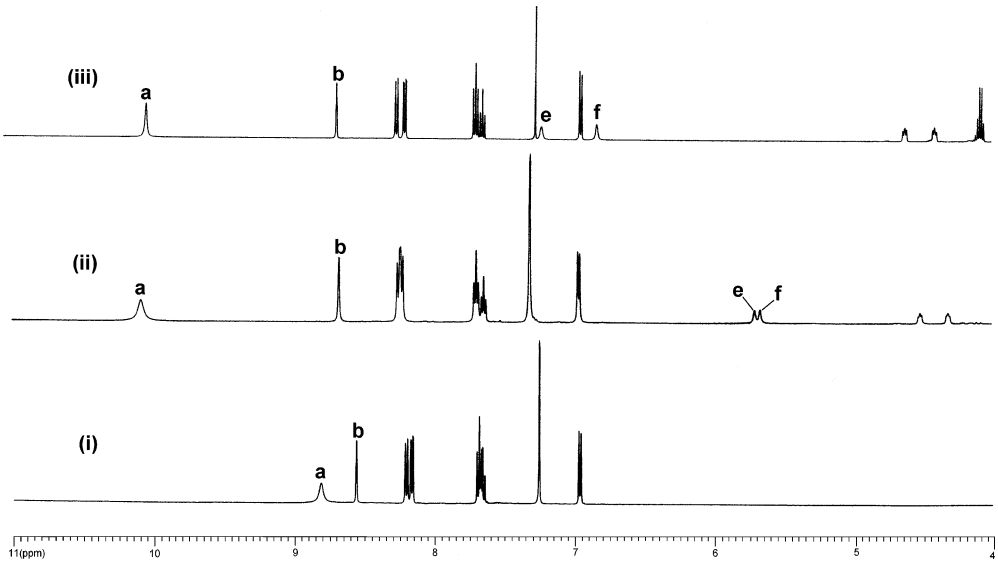

FIGURE 4. ^{1}H NMR spectra of (i) receptor **3** in $CDCl_3$, (ii) receptor **3** with biotin in 2% d_6-DMSO in $CDCl_3$, and (iii) receptor **3** with ethyl ester of biotin in $CDCl_3$ (for a, b, e, and f refer to Figure 1).

resolves the amide protons of receptor **1** as shown by the appearance of the amide peaks at two different positions, at δ 10.45 and 10.24 ppm ($\Delta\delta$ 1.51 ppm and $\Delta\delta$ 1.30 ppm), respectively. The remaining terminal pyridine amide proton is shifted from δ 8.11 to 9.20 ppm ($\Delta\delta$ 1.09 ppm) on complexation with the carboxylic acid group of biotin. So all three pyridine amide protons of receptor **1** are easily discriminated on complexation due to differential hydrogen bonding. The biotin urea protons are also actually different though it does not resolve in NMR itself (δ 5.98 ppm), but on complexation with receptor **1**, the peaks are more prominent to have distinguishable chemical shifts (δ 6.02 ppm and δ 5.90 ppm) (Table 1). In the case of biotin ethyl ester, complexation with receptor **1** in $CDCl_3$, the first two isophthaloyl amide protons have shifted their positions from δ 8.94 to δ 10.12 and δ10.18 ($\Delta\delta$ 1.18 ppm and $\Delta\delta$ 1.24 ppm) respectively but the third terminal pyridine amide proton of the receptor does not change its position. Biotin ureido protons (δ 5.26 ppm and δ 4.98 ppm) remain in the same position (δ 5.18 ppm and δ 5.08 ppm). These results suggest the hydrogen bonded complex structure of receptor **1** with biotin

TABLE 1. Chemical Shifts of Different Amide and Peri Protons of Receptors 1, 2, and 3 and Their Changes on Complexation with Biotin and Ethyl Ester of Biotin

	complex **1C**				complex **2C**			complex **3C**	
	a	b	c	peri d	a	b	peri c	a	peri b
receptor itself	8.94	8.94	8.11	8.56	9.16	8.33	8.61	8.81	8.56
with biotin	10.24	10.45	9.20	8.70	10.24	9.30	8.66	10.08	8.67
with biotin ethyl ester	10.12	10.18	8.33	8.68	9.94	8.37	8.67	9.99	8.66

is **1C**. Though final confirmation can only be obtained by single-crystal X-ray analysis, we have not yet been able to grow good quality crystals of the complex. A dummy titration was performed taking d_6-DMSO as a guest substrate to the receptors. There was no DMSO-induced chemical shift of key protons observed in any case. In the case of receptor **2**, two identical pyridine amide protons between the isophthaloyl spacer and pyridine rings appeared at the same place (δ 9.16 ppm) and shifted to δ 10.24 ppm ($\Delta\delta$ 1.08 ppm) on the event of a 1:1 complex formation with the urea part of biotin. The other two terminal pyridine acetamide protons, which appeared at δ 8.33 ppm, have shifted to δ 9.30 ppm ($\Delta\delta$ 0.97 ppm) on complexation with the carboxyl group of biotin. So this supports structure **2C** for the complex of receptor **2** with biotin. An experiment of complexation of biotin ethyl ester with receptor **2** has been performed in $CDCl_3$. Here only the amide protons nearer to the isophthaloyl group have changed their peak position from δ 9.16 ppm to δ 9.94 ppm ($\Delta\delta$ 0.78 ppm), but the other two do not change their position with biotin ethyl ester which also reaffirms the complex structure **2C** of receptor **2** with biotin. The ureido protons of biotin on complexation with receptor **2** (δ 5.98 ppm) have also exhibited more downfield shift (δ 5.89 ppm and δ 5.78 ppm, respectively), whereas the ureido protons of ethyl biotin shifted more downfield (δ 5.18 ppm to δ 6.40 ppm and δ 5.08 ppm to δ 5.99 ppm). Now receptor **3**, containing only the isophthaloyl pyridine diamide groups, can bind either the urea part or the carboxyl group of biotin. But only the urea part is bound and not the carboxyl group of biotin as proved by the NMR experiments. In NMR titration with biotin, only one pyridine amide proton is shifted from δ 8.81 ppm to δ 10.08 ppm ($\Delta\delta$ 1.27 ppm) on complexation with biotin and no other different amide proton peak has been found. An interesting result was observed here that biotin shows a significant upfield chemical shift from δ 5.98 ppm to δ 5.71 and 5.67 ppm when it was complexed with receptor **3**. Two ureido protons at δ 5.26 ppm and δ 4.98 ppm shifted downfield to δ 7.21 ppm and δ 6.83 ppm on complexation with the same receptor. In all cases, the peak of the amide protons of biotin and biotin ethyl ester has changed position toward downfield. From these observations, it is concluded that only the urea linkage of biotin is incorporated into the cavity but not that of the carboxyl group. No evidence of proton transfer to the receptor from the biotin $-CO_2H$ group is found in the solution phase. The intermolecular hydrogen bonding of carboxyl and ureido part of biotin is obviously broken by the interactions in heterocomplexation. The peri protons of the isophthaloyl moiety in receptors **1**, **2**, and **3** furnish significant downfield shifts (from δ 8.56 to 8.70 ppm, $\Delta\delta$ 0.14 ppm; δ 8.61 to 8.66 ppm, $\Delta\delta$ 0.05 ppm; δ 8.56 to 8.67 ppm, $\Delta\delta$ 0.11 ppm, respectively) on complexation with biotin and similar shifts are observed in the case of complexation with biotin ethyl ester. Interestingly, in biotin as well as biotin ethyl ester, the urea protons do not resolve in the NMR spectra, but they do resolve on complexation with all the receptors undergoing differential chemical shifts.

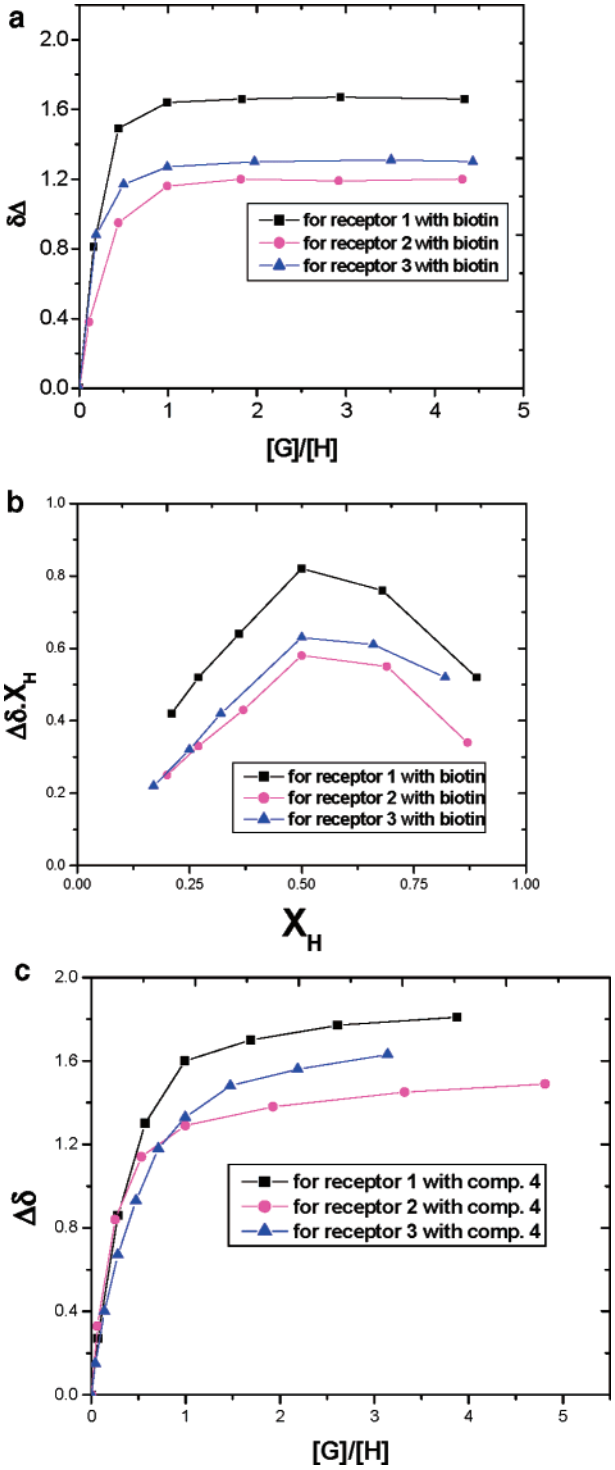

FIGURE 5. (a) Titration curves of receptors **1**, **2**, and **3** with biotin in 2% d_6-DMSO$-CDCl_3$.[14] (b) Job plots for the determination of the stoichiometry of the complexes. (c) Titration curves of receptors **1**, **2**, and **3** with biotin ethyl ester (compound **4**) in $CDCl_3$.

TABLE 2. Association Constants (K_a) of Receptors 1, 2, and 3 Respectively with Biotin and Biotin Ethyl Ester (compound 4)

	UV−Vis method K_a (M^{-1}) ($CHCl_3$)		NMR method K_a (M^{-1}) (2% d_6-DMSO−$CDCl_3$)		free energy change ΔG (kcal mol^{-1})	
receptors	with biotin	with biotin ethyl ester	with biotin	with biotin ethyl ester	with biotin	with biotin ethyl ester
receptor **1**	$4.51(\pm0.01) \times 10^4$	$3.33(\pm0.01) \times 10^4$	$1.3(\pm0.01) \times 10^4$	$1.74(\pm0.01) \times 10^3$	−5.60	−4.41
receptor **2**	$2.23(\pm0.01) \times 10^4$	$2.24(\pm0.01) \times 10^3$	$9.0(\pm0.1) \times 10^3$	$8.84(\pm0.1) \times 10^2$	−5.39	−4.01
receptor **3**	$5.23(\pm0.01) \times 10^3$	$1.95(\pm0.01) \times 10^3$	$2.4(\pm0.2) \times 10^3$	$3.18(\pm0.2) \times 10^2$	−4.60	−3.41

In electronic spectra, receptor **1** (7.843×10^{-5} mL^{-1}) exhibited absorption maxima at λ_{max} 286 nm. Addition of biotin solution to receptor **1** shows a decrease in absorption intensity. Similar results were found when the UV titrations were carried out taking receptors **2** (8.902×10^{-5} mL^{-1}) and **3** (1.734×10^{-5} mL^{-1}), respectively. Receptors **2** and **3** have shown the absorption maxima at λ_{max} 282 and 288 nm in the ground state. However, we do not find any λ-shift (blue or red) on dilution of the complexes. The gradually decreasing absorbance values (λ_{max} = 286, 282, and 288 nm, respectively) on dilution of the 1:1 complex between the receptors and biotin have been shown in all three cases in their UV spectra.[12]

We have also studied the IR spectra of biotin and receptors **1**, **2**, and **3** along with their 1:1 complexes with biotin, respectively. The biotin carbonyl group ($\nu_{C=O}$) mainly appears at 1705 cm^{-1}. On complexation of biotin with receptor **2**, the peak is shifted to 1700 cm^{-1}. In the case of receptor **3**, in complex formation with biotin a big hump is found around 1686 cm^{-1} for biotin carbonyl. A similar observation is found for receptor **1** in biotin on complexation where a broad peak for biotin carbonyl around 1680 cm^{-1} appears in the complex. The shift to lower wavenumbers of carbonyl in biotin on complexation with receptors **1**, **2**, and **3**, respectively, proves the involvement of carbonyl oxygen of biotin in the formation of hydrogen bond to form the complexes with receptors **1**, **2**, and **3**, respectively.

The energy minimized structure (CPK model)[13] of the complexes has also shown that the biotin molecule has been incorporated in the cavity of receptors **1** and **2**, respectively, and thus supports the compatible donor−acceptor points for favorable binding.[12]

Determination of Stoichiometry. From the NMR titration, the graphs [G]/[H] vs $\Delta\delta$ are plotted which show parallel straight lines with respect to the *X*-axis after 1:1 complexation, i.e., [G]/[H] = 1, for the above three receptors with biotin and biotin ethyl ester, respectively (Figure 5a,c). Figure 5b represents the Job plots (X_H vs $\Delta\delta X_H$) of the three receptors drawn from another NMR experiment, which clearly indicates the (1:1) stoichiometry of the complexes.

Quantification of Association Constants. The association constants (K_a) for the 1:1 complexes with the guest biotin have been determined by both the UV (in $CHCl_3$)[15] and NMR methods[16] (in 2% d_6-DMSO−$CDCl_3$) respectively at T = 298 K. By the NMR method, K_a values have been calculated from the δ shifts of all the NH protons of receptors **1**, **2**, and **3** (Table 2), respectively. These K_a values from the NMR (plotted $\Delta\delta$ vs $\Delta\delta$/[G]) are found to be less compared to those obtained from the UV methods (plotted 1/[G] vs $I_o/\Delta I$). In NMR, a higher concentration of the individual dimer may give rise to weaker forces of attraction resulting in smaller K_a values. The host and guest are more free in lower concentrations as used in UV and consequently the effective concentration of the 1:1 complex dimer is relatively higher in the UV studies. DMSO-d_6 used as a cosolvent in NMR studies is a polar solvent, which competes as a H-bond acceptor. So K_a values found by UV are generally higher than those determined by NMR.

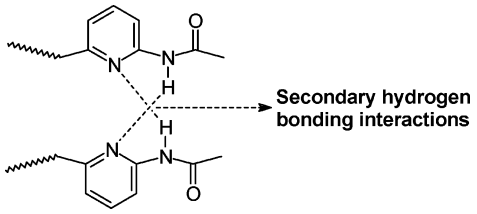

FIGURE 6. Probable secondary hydrogen bonding interactions between the two flexible pyridine amide parts of receptor **2**.

From the NMR analysis, receptor **1** shows large chemical shift changes as well as higher K_a values with biotin compared to those of receptor **2**. This may be due to the flexibility of the two aliphatic chains of receptor **2** which may come closer by secondary hydrogen bonding interactions and thus possibly create hindrance to the carboxyl group of biotin for its entry into the binding region (Figure 6).[17] However, the complexation of receptor **2** and biotin shows that the hetero-hydrogen bonding interactions are stronger than self-hydrogen bonding.

Receptor **3** has the lowest binding constant in the series as it binds only the cyclic urea part of biotin. These observed results with receptors **1**, **2**, and **3** are significant in the molecular recognition studies of biotin. The terminal pyridine amide of the receptor should be acetylamino and preferably not pivaloylamino in this case for binding biotin carboxyl group due to possible steric inhibition of the hydrogen bond by bulky pivaloylamide.

The binding constants of receptors **1**, **2**, and **3** with biotin and its ethyl ester (compound **4**) are shown in Table 2. Biotin ethyl ester binds weaker with receptors **1** and **2** compared to biotin, which suggests the formation of more hydrogen bonds of these receptors with biotin compared to biotin ethyl ester. The truncated receptor **3** also binds biotin ethyl ester with its ureido moiety.

(12) See the Supporting Information.
(13) PCMODEL Serena Software 93.
(14) Titration curve of Figure 3a,c was drawn taking the shifts of N−H[a] proton of receptor **1**, N−H[a] proton of receptor **2**, and N−H[a] proton of receptor **3** as a function of molar concentration of biotin.
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Conclusion

We thus report here the design and synthesis of receptors **1** and **2** for tandem binding of biotin. The receptors are aimed at binding simultaneously both functional groups of biotin. The binding results when compared with those of the truncated receptor **3** suggest that both receptors **1** and **2** bind stronger than **3** supporting the higher hydrogen bonding capability of **1** and **2** with biotin. Moreover the binding ability of **1** is more than that of **2** with biotin, which may be partially due to steric reasons and also due to secondary hydrogen bonding interaction. The energy minimization study also reveals the involvement of maximum hydrogen bonding in the cases of receptors **1** and **2** with biotin as shown in their complexes **1C** and **2C** respectively. The corresponding truncated receptor **3** acts as a partial receptor binding only the urea part and not the carboxyl moiety of biotin as shown in **3C**. The binding of biotin ethyl ester (compound **4**) with all these receptors **1**, **2**, and **3** is also compared.

The chiral recognition of biotin by a specific chiral receptor is underway along with the development of its fluorescent receptor which will be reported in due course.

Experimental Section

Preparation of Ethyl Ester of Biotin (compound 4). Commercially available D(+)-biotin (250 mg) was added into dry ethanol (10 mL) in a round-bottomed flask. Concentrated H_2SO_4 (two drops) was added to it and refluxed (12 h). After the usual workup, an off-white solid material was isolated (yield 79%; 220.0 mg). Mp 80−81 °C. ^{1}H NMR($CDCl_3$; 500 MHz): δ 5.26 (br s, 1H), 5.20 (br s, 1H), 4.52 (t, 1H, J = 6.2 Hz), 4.33 (t, 1H, J = 5.9 Hz), 4.13 (q, 2H, J = 7.1 Hz), 3.17 (m, 2H), 2.93 (dd, 1H, J = 5.0, 4.9 Hz), 2.74 (d, 2H, J = 12.83 Hz), 2.32 (t, 2H, J = 7.3), 1.69−1.66 (m, 2H), 1.49−1.44 (m, 2H), 1.25 (t, 3H, J = 7.11 Hz).

Preparation of *N*-{6-[6-(2,2-Dimethylpropionylamino)pyridin-2-ylmethoxymethyl]pyridin-2-yl}-2,2-dimethylpropionamide (5). To a solution of 2-(*N*-pivalylamino)-6-hydroxymethylpyridine (500 mg, 2.4 mmol) in dry tetrahydrofuran was added sodium hydride (173 mg) and the solution was stirred under nitrogen atmosphere for 1 h. The solution of 2-(*N*-pivalylamino)-6-bromomethylpyridine (650 mg, 2.4 mmol) in dry tetrahydrofuran was added dropwise to the reaction mixture under nitrogen atmosphere with stirring for 12 h. Tetrahydrofuran was removed under vacuum and dichloromethane was added to the solid. The organic layer was washed with brine solution and dried over anhydrous sodium sulfate. Then the solvent was stripped off and the residue was purified by column chromatography (using 60−120 silica gel) by using 1% methanol and chloroform to yield a brownish white semisolid **5** as reported earlier (255 mg, yield 64%).[11]

6-{[(6-Amino-2-pyridyl)methoxy]methyl}-2-pyridinamine (6). Compound **5** (0.5 g, 1.25 mmol) was dissolved in 1:1 ethanol−water (10 mL) and 4 N KOH and the solution was refluxed for 6 h. Then the solvent was removed and water was added to the residue and extracted with ethyl acetate. The organic layer was separated and dried over anhydrous sodium sulfate. The diamino compound **6** was obtained through short column chromatography with 3% methanol in chloroform. A deep brown solid material **6** was isolated (260 mg, yield 90%, mp-85 °C). ^{1}H NMR($CDCl_3$; 500 MHz): δ 7.45 (t, 2H, J = 7.77 Hz), 6.83 (d, 2H, J = 7.32), 6.40 (d, 2H, J = 8.10 Hz), 4.57 (s, 4H), 4.25 (br s, 4H).

***N*-[6-(6-Aminopyridin-2-ylmethoxymethyl)pyridin-2-yl]acetamide (7).** Compound **6** (550 mg, 2.0 mmol) was taken in dry dichloromethane (5 mL) and 0.5 equiv of acetic anhydride (1 mmol) was added to it. Then the solution was stirred at room temperature for 2 h. Compound **7** was isolated through column chromatography, using 100−200 silica gel and 1% methanol in chloroform as eluant: 230 mg, yield 42%, semisolid. ^{1}H NMR ($CDCl_3$; 300 MHz): δ 8.53 (br s, 1H), 8.11 (d, 1H, J = 8.2 Hz), 7.71 (t, 1H, J = 7.9 Hz), 7.45 (t, 1H, J = 7.7 Hz), 7.20 (d, 1H, J = 7.5 Hz), 6.80 (d, 1H, J = 7.8 Hz), 6.42 (d, 1H, J = 8.2 Hz), 4.60 (s, 4H), 4.57 (s, 2H), 2.20 (s, 3H). ^{13}C($CDCl_3$, 125 MHz): δ 169.3, 158.5, 156.7, 156.4, 151.3, 139.4, 138.9, 118.0, 113.1, 111.9, 108.1, 73.9, 73.4, 25.0. MS(ESI) (*m*/*z*, %): 295 (M + Na^+, 80%), 273 (MH^+), 139 (100). FT-IR (KBr, ν_{max}): 3336, 1682, 1539, 1456, 1369, 1302, 1158, 1103, 793 cm^{-1}.

Synthesis of Receptors 1, 2, and 3. To dichloromethane (30.0 mL) was added isophthaloyl chloride (40 mg, 0.19 mmol) with stirring. Compound **7** (100 mg, 0.36 mmol) and 2-amino-6-methylpyridine (40 mg, 0.36 mmol) were each dissolved separately in dry dichloromethane (15.0 mL). Then they were added dropwise to the isophthaloyl chloride from a high dilution dropping funnel. Addition was continued for 1 h with stirring under nitrogen atmosphere. The reaction was prolonged for 12 h. Receptors **1**, **2**, and **3** were isolated through preparative TLC, using methanol (5%) in dichloromethane.

Biotin: ^{1}H NMR (d_6-DMSO; 500 MHz)**:** δ 5.98 (br s, 2H), 4.46 (t, 1H, J = 5.1 Hz), 4.28−4.26 (m, 1H), 3.15 (m, 2H), 2.88 (dd, 2H, J = 5.0, 5.0 Hz), 2.74 (d, 2H, J = 12.70 Hz), 2.28 (t, 2H, J = 7.4 Hz), 1.66−1.62 (m, 2H), 1.45 (t, 1H, J = 7.6 Hz).

***N*-[6-(6-Acetylaminopyridin-2-ylmethoxymethyl)pyridin-2-yl]-*N*′-(6-methylpyridin-2-yl)isophthalamide (Receptor 1).** Mp 86−87 °C. Isolated yield 17%. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 8.94 (br s, 2H), 8.56 (s, 1H), 8.30 (d, 1H, J = 8.2 Hz), 8.19 (d, 1H, J = 8.2 Hz), 8.16 (d, 2H, J = 7.7 Hz), 8.11 (br s, 1H), 7.79 (t, 1H, J = 7.8 Hz), 7.72 (d, 1H, J = 7.8 Hz), 7.69−7.64 (m, 3H), 7.24 (d, 1H, J = 7.4 Hz), 7.18 (d, 1H, J = 7.4 Hz), 6.96 (d, 1H, J = 7.4 Hz), 4.64 (s, 2H), 4.62 (s, 2H), 2.46 (s, 3H), 2.16 (s, 3H). ^{13}C NMR ($CDCl_3$; 125 MHz): δ 169.2, 165.1, 165.1, 157.3, 156.7, 156.4, 151.4, 151.4, 151.2, 139.6, 139.5, 139.3, 135.3, 135.1, 131.5, 131.4, 129.8, 126.3, 120.1, 118.3, 117.9, 113.5, 113.3, 111.6, 73.5, 73.4, 25.0, 24.3. MS (FAB) (*m*/*z*, %): 533 (M + Na^+, 20), 511 (MH^+, 100), 467 (18), 439 (30), 242 (80), 154 (80). FT-IR (KBr, ν): 2360, 1682, 1577, 1540, 1455, 1303, 797 cm^{-1}. Anal. Calcd for $C_{28}H_{26}N_6O_4$ (510.56): C, 65.87; H, 5.13; N, 16.46; O, 12.53. Found: C, 65.89; H, 5.14; N, 16.46.

Complex of Receptor 1 with Biotin. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 10.46 (br s, 1H), 10.24 (br s, 1H), 9.20 (br s, 1H), 8.70 (s, 1H), 8.32 (d, 1H, J = 8.2 Hz), 8.26 (t, 2H, J = 7.2 Hz), 8.22 (d, 1H, J = 8.1 Hz), 8.11 (d, 1H, J = 7.8 Hz), 7.79 (t, 1H, J = 7.9 Hz), 7.74−7.70 (m, 2H), 7.64 (t, 1H, J = 7.7 Hz), 7.26 (d, 1H, J = 7.4 Hz), 7.18 (d, 1H, J = 7.4 Hz), 6.99 (d, 1H, J = 7.4 Hz), 6.02 (br s, 1H), 5.90 (br s, 1H), 4.69 (s, 2H), 4.64 (s, 2H), 4.49−4.47 (m, 1H), 4.28−4.26 (m, 1H), 2.88 (dd, 2H, J = 5.0, 5.0 Hz), 2.53 (s, 3H), 2.46 (d, 1H, J = 4.4 Hz), 2.29 (t, 2H, J = 7.4 Hz), 2.18 (s, 3H), 1.66−1.58 (m, 2H), 1.49−1.41 (m, 2H), 0.87 (t, 2H, J = 6.8 Hz).

Complex of Receptor 1 with the Ethyl Ester of Biotin. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 10.18 (br s, 1H), 10.12 (br s, 1H), 8.68 (s, 1H), 8.35 (br s, 1H), 8.32 (d, 1H, J = 8.3 Hz), 8.24 (d, 1H, J = 8.2 Hz), 8.20 (d, 2H, J = 7.4 Hz), 8.10 (d, 1H, J = 7.9 Hz), 7.77 (t, 1H, J = 7.9 Hz), 7.70−7.66 (m, 2H), 7.64 (t, 1H, J = 7.7 Hz), 7.17 (d, 1H, J = 7.4 Hz), 7.10 (d, 1H, J = 7.5 Hz), 6.96 (d, 1H, J = 7.4 Hz), 5.18 (br s, 1H), 5.08 (br s, 1H), 4.59 (s, 4H), 4.53−4.50 (m, 1H), 4.32−4.30 (m,1H), 4.12 (q, 2H, J = 7.1 Hz), 3.18−3.14 (m, 1H), 2.92 (dd, 1H, J = 5.0, 5.0 Hz), 2.73 (d, 1H, J = 12.8 Hz), 2.46 (s, 3H), 2.31−2.27 (m, 2H), 2.12 (s, 3H), 1.69−1.66 (m, 2H), 1.49−1.38 (m, 2H), 1.25 (t, 3H, J = 7.0 Hz), 0.88 (t, 2H, J = 6.7 Hz).

***N*,*N*′-Bis[6-(6-acetylaminopyridin-2-ylmethoxymethyl)pyridin-2-yl]isophthalamide (receptor 2).** Mp 167−69 °C; isolated yield 22%. ^{1}H NMR ($CDCl_3$; 500 MHz) δ 9.17 (br s, 2H), 8.61 (s, 1H), 8.34 (br s, 2H), 8.29 (d, 2H, J = 8.2 Hz), 8.19 (d, 2H, J = 7.7 Hz), 8.08 (d, 2H, J = 7.7 Hz), 7.78 (t, 2H, J = 7.8 Hz), 7.68 (t, 1H, J = 7.9 Hz), 7.64 (d, 2H, J = 7.7 Hz), 7.21 (d, 2H, J = 7.4 Hz), 7.14 (d, 2H, J = 7.4 Hz), 4.60 (s, 4H), 4.59 (s, 4H), 2.15 (s, 6H).

^{13}C NMR ($CDCl_3$; 125 MHz): δ 169.7, 156.6, 156.4, 151.9, 151.5, 139.4, 134.8, 132.1, 131.9, 129.7, 126.5, 119.8, 118.2, 117.8, 113.8, 113.2, 111.8, 73.5, 24.9. MS (ESI) (*m*/*z*, %): 697 ($M + Na^+$, 15), 675 (MH^+, 100), 525 (20), 476 (30). FT-IR (KBr, ν): 2926, 2360, 1680, 1601,1578, 1536, 1455, 1302, 1235, 796, 721 cm^{-1}. Anal. Calcd for $C_{36}H_{34}N_8O_6$ (674.72): C, 64.09; H, 5.08; N, 16.61; O, 14.23. Found: C, 64.10; H, 5.08; N, 16.62.

Complex of Receptor 2 with Biotin. ^{1}H NMR (2% d_6-DMSO + $CDCl_3$; 500 MHz): δ 10.25 (br s, 2H), 9.30 (br s, 2H), 8.66 (s, 1H), 8.31 (d, 2H, J = 8.2 Hz), 8.22 (d, 2H, J = 7.7 Hz), 8.10 (d, 2H, J = 7.4 Hz), 7.80 (t, 2H, J = 7.9 Hz), 7.70 (t, 2H, J = 7.8 Hz), 7.64 (t, 1H, J = 7.7 Hz), 7.26 (d, 2H, J = 7.4 Hz), 7.18 (d, 2H, J = 7.4 Hz), 5.89 (br s, 1H), 5.79 (br s, 1H), 4.66 (s, 4H), 4.62 (s, 4H), 4.48−4.45 (m, 1H), 4.27−4.25(m, 1H), 3.19−3.18 (m, 1H), 2.87 (dd, 2H, J = 5.0, 5.0 Hz), 2.73 (d, 1H, J = 12.7 Hz), 2.29 (t, 2H, J = 7.3 Hz), 2.18 (s, 6H), 1.67−1.61 (m, 3H), 1.48−1.42 (m, 2H).

Complex of Receptor 2 with Ethyl Ester of Biotin. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 9.94 (br s, 2H), 8.67 (s, 1H), 8.37 (br s, 2H), 8.32 (d, 2H, J = 8.2 Hz), 8.20 (d, 2H, J = 8.9 Hz), 8.10 (d, 2H, J = 7.6 Hz), 7.78 (t, 2H, J = 7.8 Hz), 7.71−7.61 (m, 3H), 7.18 (d, 2H, J = 7.4 Hz), 7.11 (d, 2H, J = 7.4 Hz), 6.40 (br s, 1H), 5.99 (br s, 1H), 4.58 (s, 4H), 4.56 (s, 4H), 4.44−4.42 (m, 1H), 4.19−4.17 (m, 1H), 4.10 (q, 2H, J = 6.8 Hz), 3.10−3.08 (m, 1H), 2.82 (dd, 2H, J = 5.0, 4.9 Hz), 2.70 (d, 1H, J = 12.7 Hz), 2.46−2.44 (m, 2H), 2.31−2.26(m, 2H), 2.23 (t, 2H, J = 7.5 Hz), 2.19 (s, 3H), 2.15 (s, 3H), 1.60−1.55 (m, 2H), 1.26 (t, 2H, J = 6.0 Hz).

***N,N′*-Bis(6-methylpyridin-2-yl)isophthalamide (receptor 3).** Isolated yield 26%. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 8.81 (brs, 2H), 8.56 (s, 1H), 8.21 (d, 2H, J = 7.7 Hz), 8.17 (d, 2H, J = 7.7 Hz), 7.70−7.64 (m, 3H), 6.97 (d, 2H, J = 7.5 Hz), 2.49 (s, 6H).

Complex of Receptor 3 with Biotin. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 10.08 (br s, 2H), 8.67 (s, 1H), 8.26−8.22 (m, 4H), 7.69 (t, 2H, J = 7.8 Hz), 7.64 (t, 1H, J = 7.7 Hz), 6.97 (d, 2H, J = 7.4 Hz), 5.71 (br s, 1H), 5.67 (br s, 1H), 4.52 (t, 1H, J = 6.3 Hz), 4.34 (t, 1H, J = 5.5 Hz), 3.16 (t, 1H, J = 6.7 Hz), 2.92 (dd, 2H, J = 5.0, 4.8 Hz), 2.74 (d, 1H, J = 12.8 Hz), 2.52 (s, 6H), 2.30 (t, 2H, J = 6.5 Hz), 1.69−1.62 (m, 2H), 1.46 (t, 3H, J = 6.9 Hz).

Complex of Receptor 3 with Ethyl Ester of Biotin. ^{1}H NMR ($CDCl_3$; 500 MHz): δ 9.99 (br s, 2H), 8.66 (s, 1H), 8.23 (d, 2H, J = 8.3 Hz), 8.18 (dd, 2H, J = 1.7, 1.7 Hz), 7.67 (t, 2H, J = 7.8 Hz), 7.62 (t, 1H, J = 7.7 Hz), 7.21 (br s, 1H), 6.94 (d, 2H, J = 7.4 Hz), 6.83 (br s, 1H), 4.65−4.62 (m, 1H), 4.43−4.41 (m, 1H), 4.09 (q, 2H, J = 7.2 Hz), 3.21−3.16 (m, 1H), 2.95 (dd, 2H, J = 5.1, 5.1 Hz), 2.76 (d, 1H, J = 12.5 Hz), 2.47 (s, 6H), 2.26 (t, 2H, J = 7.1 Hz), 1.62−1.57 (m, 2H), 1.48−1.40 (m, 3H), 1.23 (t, 3H, J = 7.1 Hz).

Experimental Procedure for Scheme 2: Synthesis of Receptors 2 and 3. Isophthaloyl chloride (70 mg, 0.34 mmol) was taken in dichloromethane (10 mL). Compound **7** (200 mg; 0.68 mmol) mixed with 0.25 mL of triethylamine was also dissolved in dry dichloromethane (15.0 mL) and taken in a dropping funnel. Then it was added dropwise to the isophthaloyl chloride solution and addition was continued for 1 h with stirring under nitrogen atmosphere. The reaction was prolonged for 12 h. Receptor **2** was isolated through preparative TLC, using methanol (5%) in dichloromethane.

The same procedure was followed to prepare receptor **3** taking 2-amino-6-methylpyridine instead of compound **7**.

Acknowledgment. We thank DST, DST-FIST, and CSIR [Project No. 01(1913)/04/EMR-II], Government of India, for financial support and appreciate mass spectral help from Professor Thomas Schrader, University of Marburg, Germany.

Supporting Information Available: NMR spectra, IR spectra, UV titration spectra of receptors and the complexes and the association constants calculation curves (both NMR and UV method), and energy minimized structures. This material is available free of charge via the Internet at http://pubs.acs.org.

JO061013J