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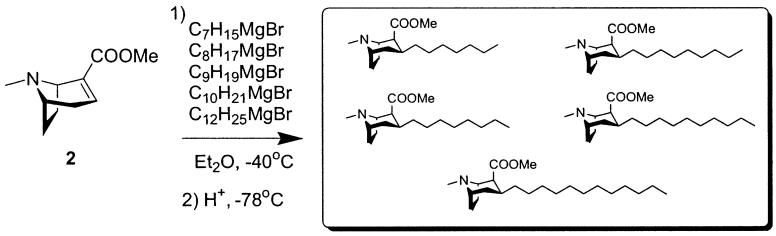

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Two- and Three Dimensional Combinatorial Chemistry from Multicomponent Grignard Reagents

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The conjugate addition of five component Grignard reagents to methyl ecgonidine was used to create libraries of 3-substituted tropanes. By variation in the reagent combination in 10 such 5-membered sublibraries, a library of 25 compounds was made in a two-dimensional format. Screening of this library led to identification of two new potent monoamine transporter ligands that were subsequently synthesized. The most potent compound in this library was (1*R*,2*S*,3*S*,5*S*)-3-(3,4-dimethylphenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester, which inhibited dopamine transporter (hDAT) binding and reuptake with a K_i of 26 and 20 nM, respectively. The conjugate addition to a 5-membered library of methyl ecgonidine analogues with variation of nitrogen substituent was also carried out and used to create 15 sublibraries of 25 compounds, which displayed 125 compounds in a three-dimensional format. From this 3D library, several potent dopamine transport inhibitors were likewise identified and synthesized. The most potent hDAT inhibitor discovered was (1*R*,2*S*,3*S*,5*S*)-3-(3,4-dimethylphenyl)-8-pentyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester. The study also showed that 3-alkyltropanes were poor inhibitors of monoamine transporters.

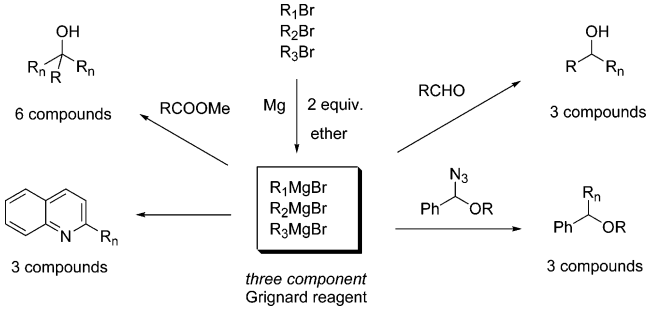
Introduction

Combinatorial chemistry represents a range of techniques, some automated, that permits the rapid synthesis of a large number of chemical compounds usually through the "combination" of several synthetic building blocks.[1,2] Growing out of peptide chemistry, the combinatorial chemistry of peptides and peptide-like molecules is very effective.[3,4] Many other compound types, such as enzyme inhibitors, carbohydrates, and catalysts, have been successfully approached using solid-[5,6] or solution-phase[7,8] combinatorial methods. Nevertheless, the usefulness of combinatorial methods is measured against whether they are reliable and easy enough to carry out to replace the traditional investigation of individual compounds, possibly through so-called parallel synthesis, and these demands are in many cases difficult to meet. Therefore, methods that allow the reliable screening of many drug analogues are still in high demand. On the other hand, with such methods available, the drug discovery process will unquestionably be significantly advanced.

Recently it was found that multicomponent Grignard reagents can be made and reacted with several different electrophiles to generate uniform mixtures of products.[9−11] Using this technology, libraries of alkylated products are, indeed, very easily generated and can be screened (Scheme 1).

However, the identification of the biologically active library members would normally require individual synthesis of each compound in an active library or so-called deletion synthesis.[12] To avoid the unnecessary synthesis of scores of inactive compounds, we here report a method that allows a very facile identification of active compounds from libraries generated from multicomponent Grignard reagents. We illustrate the method in the search and discovery of potential cocaine antagonists.

Scheme 1. The Preparation of Multicomponent Grignard Reagents and Their Use to Synthesize Libraries of Secondary and Tertiary Alcohols, Ethers and 2-Substituted Quinolines



In the method, the compound set is resolved into dimensions so that compounds can be identified similarly to positional scanning[7] or indexed libraries.[13,14] However, since these methods require either as many reaction sites as dimensions or a variation of both reaction partners, they are not directly applicable to the reaction of many reagents with one compound. It was suggested that variable mixing of the Grignard reagents could be used to achieve the desired dimensions (Figure 1). To prepare n^2 compounds, n libraries with n compounds would be normaly prepared, and each product or Grignard reagent can be assigned a coordinate x, y, where x is the library number and y, the number of the

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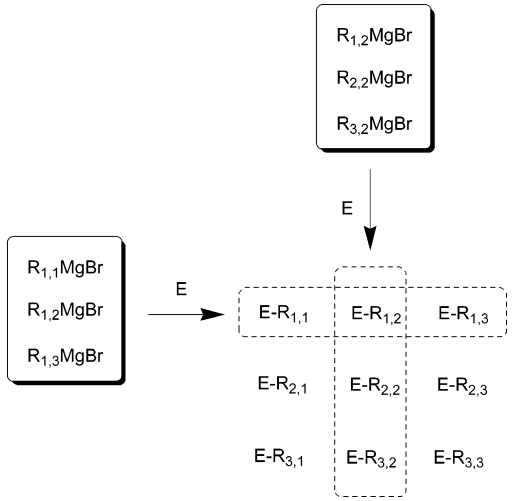


Figure 1. The synthesis of nine compounds in two dimensions using variable mixing. Six three-component Grignard reagents are reacted with an electrophile (E) to give six libraries. Screening of libraries 1,x and x,2 will show whether compound E-$R_{1,2}$ is significantly more active than the background.

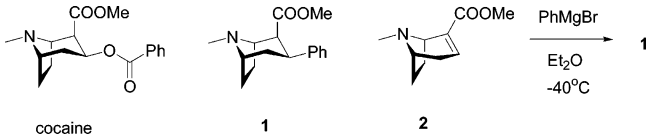


Figure 2. The cocaine analogue phenyltropane **1**, which was chosen as target, and positive control in the present work.

member. However, if the synthesis is repeated with the meaning of *x* and *y* reversed (so *y* depicts library number etc), *n* new libraries will result, containing the same n^2 compounds. By screening the 2*n* libraries, library members with extraordinary activities will be directly revealed through the display of activity in any of their coordinate libraries (Figure 1).

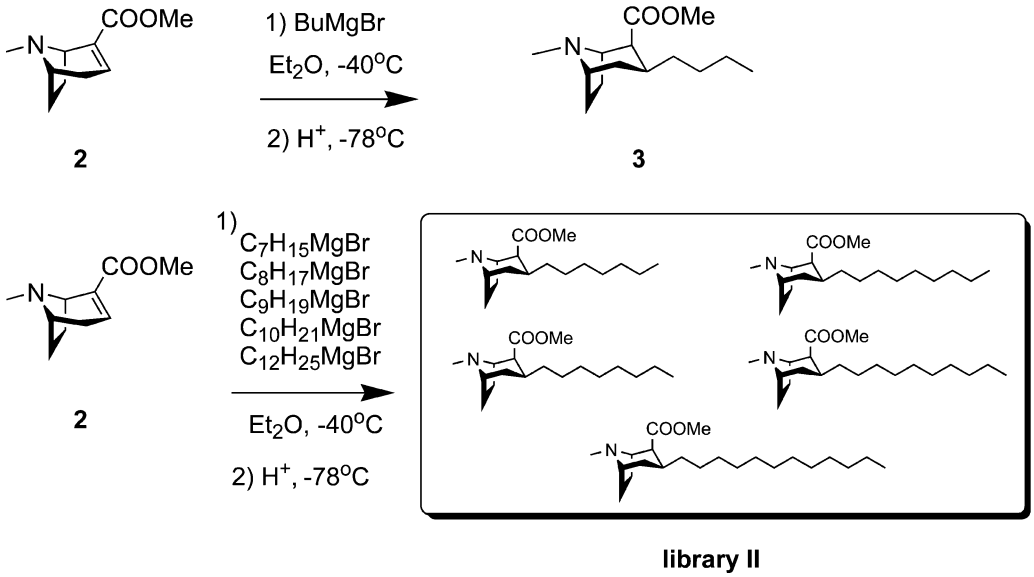
As target molecules for our combinatorial synthesis, 3-substituted tropane analogues were found suitable because (a) 3-phenyl tropanes, such as **1**, are highly potent dopamine transport inhibitors and potential cocaine antagonists,[15] and (b) phenyl tropanes are made by a 1,4-conjugated addition of Grignard reagents to methyl ecgonidine **2** (Figure 2).[16] More than 20 aryl tropanes have been prepared and investigated using this and other synthetic methods; however remarkably, not a single aliphatic substituted tropane has been reported. Accordingly, it was worthwhile to prepare and screen a larger group of particularly aliphatic 3-substituents, and it appeared a suitable testing ground for the above-mentioned technique.

We here report the results of this study, which demonstrate the power of the multicomponent Grignard technology. We show that two and three-dimensional combinatorial libraries can readily be prepared and allow rapid investigation of large sets of compounds.

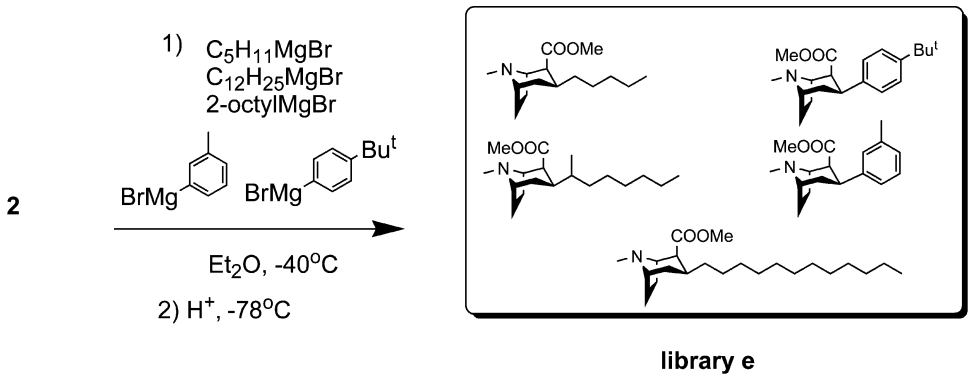
Results and Discussion

Initially, the conjugate addition to **2**[17] was studied. The successful reaction of butyl magnesiumbromide with **2** demonstrated the at that point by no means trivial observation that nonaromatic reagents could be applied in this reaction (Scheme 2). The 2β,3β-isomer **3** was obtained in high yield (76%), with only trace amounts of 2α,3β-isomer being present as a result of the lack of complete selectivity in the kinetic protonation. The stereochemistry of these compounds was determined from the ^{1}H NMR spectra. The axial H4 proton (at δ 1.79) gave two large and one small couplings, which is consistent only with the 3β stereochemistry. Second, the major isomer had the N-Me signal 0.2 ppm more upfield than the minor, which is a known effect of having the ester group axial. Next a five-component Grignard reaction was carried out using linear aliphatic bromides having from 7 to 12 carbons (Scheme 2). The reagent was prepared by sequential addition of halides to excess magnesium, as previously described[9], and the reaction was otherwise performed in a manner similar to the synthesis of **3**, except that the Grignard reagent was added slowly using a syringe pump. The product library was, similarly to all other libraries reported here, analyzed by NMR, ESMS, and GC−MS. While NMR in the present case was powerless to resolve the different aliphatic chains, both electrospray and

Scheme 2. Conjugate Addition of Butyl Magnesiumbromide and a Five-Component Reagent to Methyl Ecgonidine (**2**)



Scheme 3. Conjugate Addition of a Mixture of Aromatic and Aliphatic Reagents to Methyl Ecgonidine (**2**)



GC−MS revealed the presence of all products and indicated their presence in roughly identical amounts (see Supporting Information). A similar experiment, carried out with a mixture of aliphatic and aromatic bromides (Scheme 3), could be analyzed by NMR. Here, NMR revealed that the aromatic compounds constituted ∼40% of the library. Also here, ESMS and GC−MS confirmed the presence of all compounds.

With the success of the combinatorial reaction established, we proceeded to prepare a two-dimensional library with 25 compounds in the form of 2 × 5 sublibraries of 5 compounds. The contents of the libraries made is shown as rows (libraries I−V) and columns (libraries a−e) in the matrix in Figure 3. The halides that were finally used were chosen from a larger set that was investigated for addition to **2** as members of multicomponent Grignard reagents. A number of the halides in this larger set failed to give discernible products in the MS analysis and were therefore discarded. The multicomponent method therefore appears to be a valuable method to screen reagent reactivity, as well. On the other hand, several products from secondary aliphatic Grignard reagents were formed, such as the potentially very interesting cycloalkyl derivatives IIIa−IIId.

In the synthesized compound set (Figure 3), the presence of all library members was confirmed by GC−MS or MS (see the Supporting Information). The only known library members, Va (**1**) and IIIe (**7**), were included because (a) **1** is a potent dopamine transport inhibitor,[17] which was used as a positive control; and (b) no biological data had been disclosed for **7**.[18]

The 10 sublibraries were screened for inhibition of the monoamine transporters hDAT, hSERT, and hNET in a competitive binding assay with radiolabeled RTI-55[19] and also for the monoamine uptake using cells expressing the three transporters and radiolabeled dopamine or serotonin. The average of three experiments gave K_i values for each

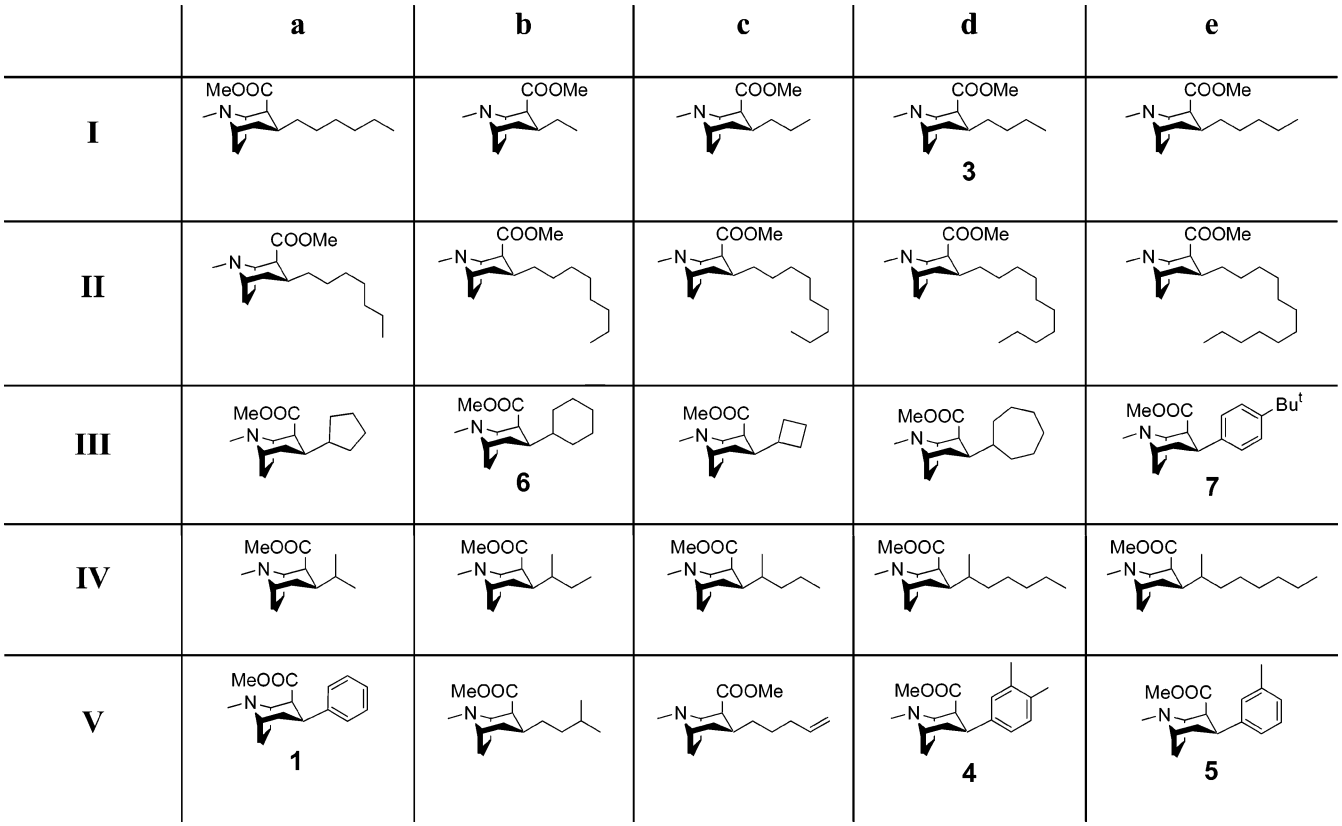


Figure 3. Contents of the two-dimensional library contained in the sublibraries I−V (rows) and a−e (columns).

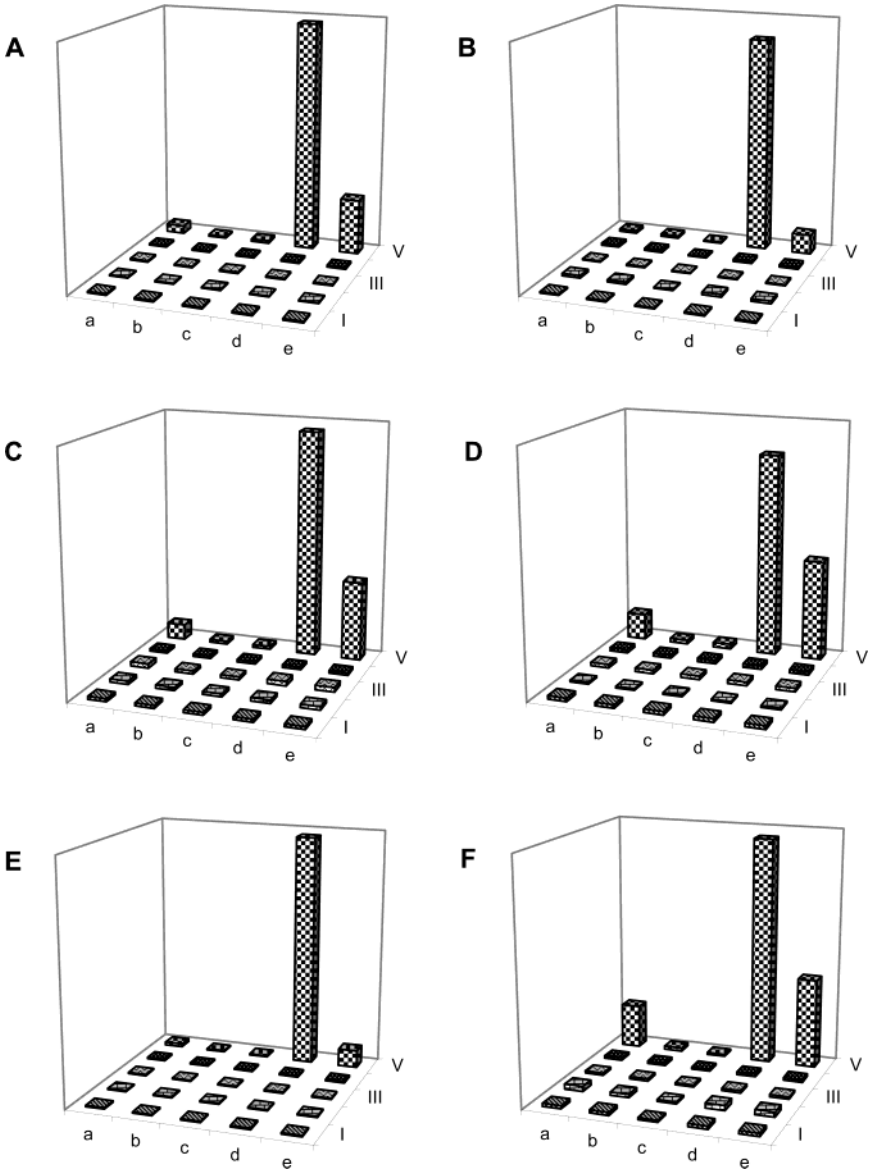


Figure 4. Graphical displays showing for each library member the smallest value of $5/K_i$ for each of the two sublibraries in which it appears. (K_i is the average value for a library.) This value is the highest possible K_a (μM^{-1}) that a library member can have. Part A shows hDAT binding, B shows hSERT binding, C shows hNET binding, D shows inhibition of hDAT-mediated DA uptake, E shows inhibition of hSERT-mediated SER uptake, and F shows inhibition of hNET-mediated DA uptake.

sublibrary (for list of K_i values, see the Supporting Information). For each library member, its highest possible association constant, K_a, was determined as the smallest $5/K_i$ value of each of the two sublibraries in which it appeared. This value was plotted as a bar in the graphical displays shown in Figure 4. From these displays, the maximum activity of the library members can be directly read. On the other hand, false hits can appear when two or more potent compounds are present in different columns and rows.

It is seen that two library members, **4** (Vd) and **5** (Ve), have above average activity in all six assays (Figure 4). In addition, the positive control **1** (Va) shows activity against the transporters, especially against hDAT and hNET, as expected.[15] Furthermore, **4** and **5** proved to be more potent inhibitors than the positive control **1**, with **4** being the more potent. All compounds with aliphatic 3-substituents were found to have low activity. To check the validity of these results, a selection of individual compounds, which included active and inactive compounds, where synthesized. The chosen compounds, **3** (Id), **4** (Vd), **5** (Ve), **6** (IIIb) and **7** (IIIe), were tested in comparison to control **1** (Table 1). These data confirmed the findings from the displays and, thus, its reliability. The aromatic compounds **1**, **4**, and **5** are 100−1000-fold more potent than the corresponding aliphatic compounds.

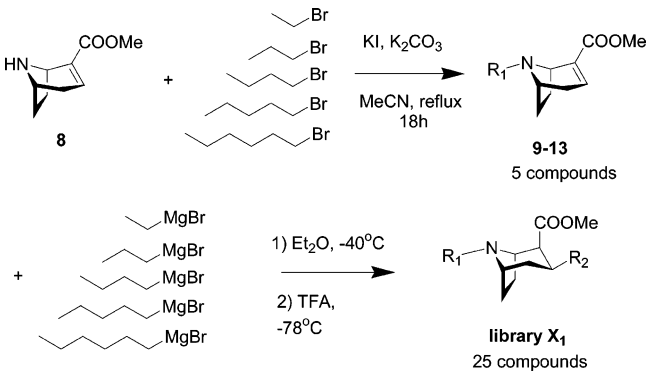
The high potency of **4** and **5** is in accordance with the presence of an aromatic 3-substituent. The increase in activity, as compared to the unsubstituted compound **1**, is similar to that observed when chlorine is introduced into the 3′- and 4′-positions in phenyl tropanes.[20] This is probably because methyl can imitate chlorine's lipophilic effect due to the similar size of Cl and methyl.

However, in contrast to this is the very poor binding displayed by the aromatic *tert*-butyl analogue **7**. The *tert*-butyl group is comparable in size to an iodine atom, and 4′-iodine-substituted phenyltropane (RTI-55) is a very potent

Table 1. K_i Values for Binding and Uptake at HDAT, HSERT, or HNET

	K_i binding (nM)			K_i uptake (nM		
compd	hDAT	hSERT	hNET	hDAT	hSERT	hNET
1	220 ± 95	750 ± 680	555 ± 455	112 ± 68	614 ± 208	115 ± 30
3	6900 ± 650	23100 ± 7300	4700 ± 650	1900 ± 750	24900 ± 3100	2650 ± 750
4	19 ± 10	15 ± 6	20 ± 7	14 ± 7	18 ± 11	13 ± 5
5	115 ± 45	250 ± 50	190 ± 110	65 ± 40	83 ± 20	40 ± 28
6	21 000 ± 4500	27 000 ± 7500	10 500 ± 3000	5300 ± 2600	36 000 ± 23 000	4100 ± 2500
7	38 000 ± 5250	3700 ± 620	29 950 ± 1300	11700 ± 1850	4150 ± 2950	15 700 ± 3300

Scheme 4. Combinatorial Synthesis of Three-Dimensional Library



compound.[21] However, since **7** in terms of electronic and lipohilic effects is quite similar to the potent compound **4**, the somewhat larger sized *tert*-butyl, as compared to I, appears to be the reason for the drastic decrease in binding affinity for all three monoamine transporters.

Finally, the displays clearly show that a wide range of 3-alkyltropanes are poor binders to monoamine transporters. Particularly remarkable is the very poor activity of cyclobutyl, cyclopentyl, and cyclohexyl, which in terms of size and lipophilicity mimic the benzene ring. Because adding more dimensions to the library increases the number of compounds produced and the potential time gain, we tried to increase the library to three-dimension by including variations at the nitrogen atom. It was decided to include these variations through attempting the conjugated addition of Scheme 2 on a library of five *N*-substituted compounds. Therefore, multicomponent alkylation of amine **8**[22] was investigated (Scheme 4), and this reaction was found to be quite successful. Thus, reaction of **8** with five homologous alkyl bromides in the presence of KI and K_2CO_3 gave a good yield of products with an equal ratio of the homologues **9**−**13**, as witnessed by GC−MS (see Supporting Information). Subsequent conjugate addition with a five-component Grignard reagent (library X_1, Scheme 4) gave a product that gave GC−MS and MS data consistent with the presence of 25 compounds (Figure 5). The ESMS show all the expected masses and the expected Gausian-like increase in intensity for the more abundant masses. The GC−MS shows over 15 product peaks, several with the abundant masses. The probability is therefore high that all the expected compounds were present.

With this methodology established, we proceeded to prepare 15 libraries of 25 compounds representing the 125 compounds of the cube of Figure 6 three times. Ten libraries, numbered X_1−X_5, and Y_1−Y_5, were made according to the reaction of Scheme 4, but by variation of the Grignard reagents in a manner similar to what was done in the two-dimensional library. The last five libraries, numbered Z_1−Z_5 and each representing a layer in the cube, were made by reaction of single N-substituted compound (**9**, **10**, **11**, **12**, or **13**) with a 25-component Grignard reagent made from the entire set of bromides.

The libraries X_1−X_5, Y_1−Y_5, and Z_1−Z_5 were tested for binding and inhibition of uptake on the three monoamine transporters (for a list of K_i values, see the Supporting Information). For each of the six assays, determination of the smallest value of $25/K_i(x)$, $25/K_i(y)$ or $25/K_i(z)$ for each of the 125 cube entries and plotting these gave the six plots in Figure 7. Each bar represents the maximum possible association constant, K_a, for each of the 125 compounds.

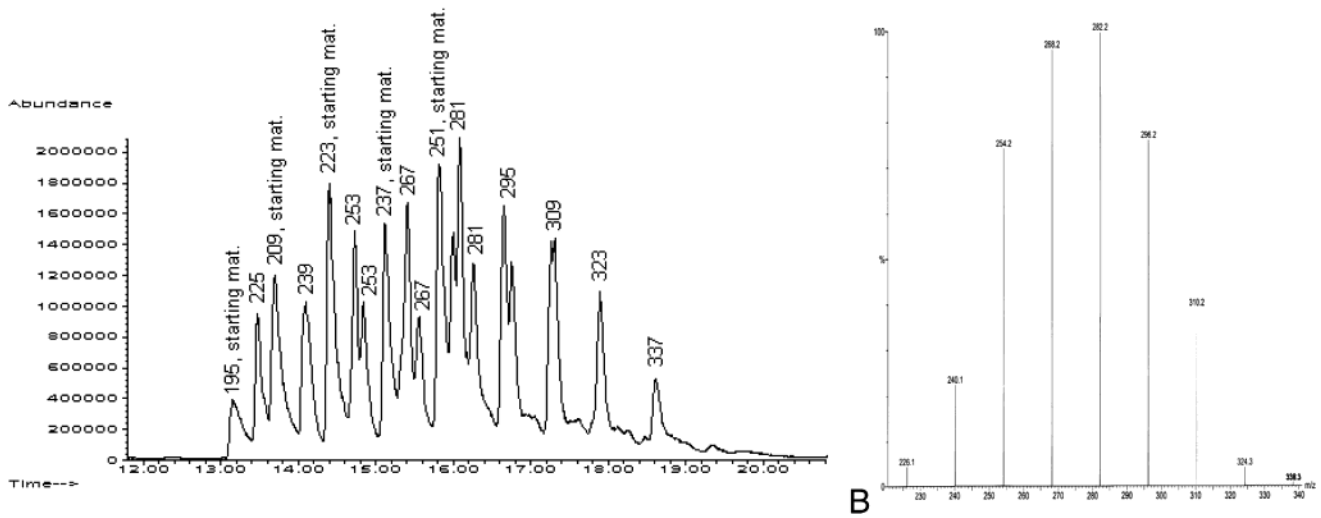


Figure 5. GC−MS (A) and ESMS (B) of library X_1.

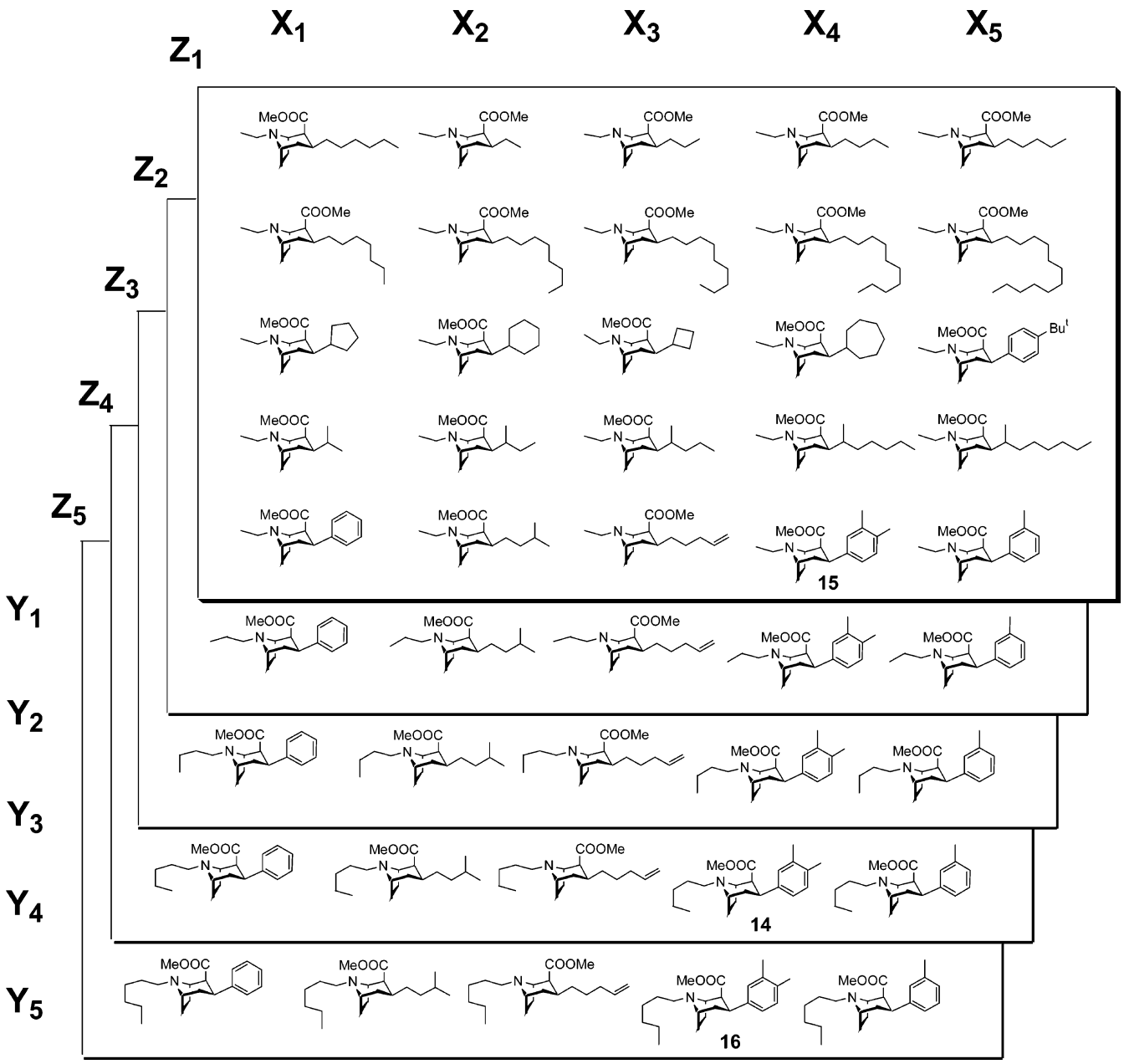


Figure 6. Three-dimensional library.

Figure 7 shows several compounds with a much higher potency than the basic threshold in the various assays. Thus the *N*-pentyl-3-dimethyl analogue **14** (4,5,4) appears to be particularly potent and the strongest inhibitor of DAT binding. The *N*-ethyl-3-dimethylphenyl analogue **15** (4,5,1) appears to be the strongest inhibitor of NET binding, while the *N*-hexyl-3-dimethyl analogue **16** (4,5,5) appears most potent in the SERT binding assay. Compounds **14** and **16** likewise appear exceptional in inhibition of uptake for all three transporters. Similar to the screening of the two-dimensional library, screening of the three-dimensional library confirms that aromatic groups in the 3β position are essential and that the 3,4-dimethylphenyl group is the best 3-substituent for inhibition of all three monoamine transporters.

Compounds **14**−**16** were selected for individual synthesis. The binding and inhibition of uptake of these three compounds were determined and are shown in Table 3. The K_i values largely reflect the observations in Figure 7: all three compounds are very potent against the DAT and have a K_i of binding similar to that of the *N*-methyl analogue **4**. In addition, **16** is found to have the most potent binding to NET among the compounds, as seen in Figure 7. However the *N*-ethyl analogue **15** is not, as in Figure 7, found to be the strongest binder to NET in the series. When comparing the series **4** and **14**−**16**, it is seen that binding to SERT decreases with the length of the nitrogen substituent.

In summary, multicomponent Grignard reagents combined with two- and three-dimensional screening, as presented here, is a reliable and powerful method for rapid screening of homologous compounds. The method is excellent for the screening of many analogues or substituent combinations that would otherwise not be made due to assumed similarity to known compounds. The method saves both in the time spent on synthesis and in the effort made on testing the compounds. From a synthesis standpoint, the method can be applied to

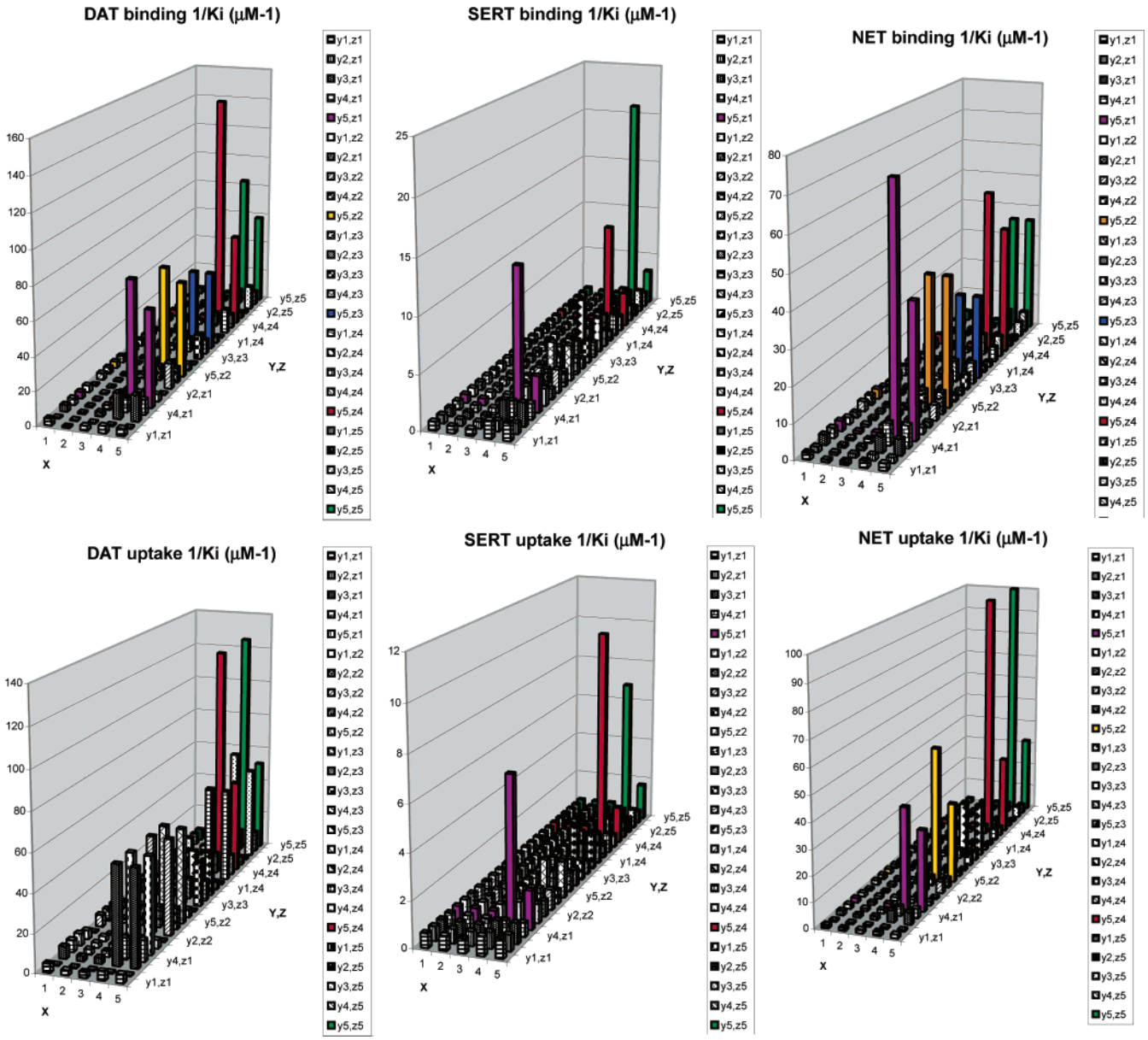
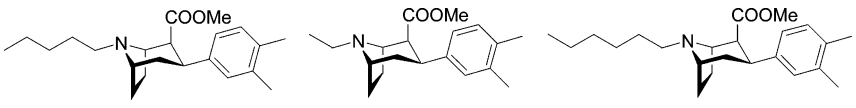


Figure 7. K_a values (μM^{-1}) for binding and uptake at hDAT, hSERT, or hNET for each library member. These values are obtained as the smallest value of $25/K_i$ for the three libraries in which the member appears and represent the highest possible K_i value.

Table 2. K_i Values for Binding and Uptake at hDAT, hSERT or hNET

	K_i binding (nM)			K_i uptake (nM)		
compd	hDAT	hSERT	hNET	hDAT	hSERT	hNET
14	14 ± 3	220 ± 15	345 ± 230	34 ± 10	69 ± 33	72 ± 38
15	18 ± 5	133 ± 50	165 ± 89	42 ± 13	78 ± 31	59 ± 26
16	25 ± 6	297 ± 82	580 ± 140	140 ± 44	310 ± 64	285 ± 64



14 (x,y,z = 4,5,4) **15** (x,y,z = 4,5,1) **16** (x,y,z = 4,5,5)

Figure 8. Hits in the three-dimensional library. Compound **14** is the strongest hit against hDAT, **15** is more potent against hSERT, and **16** is the strongest compound binding to hNET.

the preparation of vastly larger libraries, but larger libraries will only be meaningful to screen if a very large differences in biological activity between exceptional and unexceptional library members are expected.

Experimental Section

General Procedure for Preparation of Grignard Reagents. *Preparation of Individual Grignard Reagent.* An

alkyl or aryl bromide (10 mmol) was added dropwise to Mg (20 mmol) in 10 mL of dry diethyl ether to keep the mixture refluxing. After addition, the mixture was stirred under reflux for 2 h. Before being taken out via a syringe and added to the electrophile, the Grignard reagent was titrated according to a known procedure.[23]

Preparation of Multicomponent Grignard Reagents. Five different alkyl or aryl bromides were employed in equimolar amounts (2 mmol each). They were added slowly one at a time to Mg (20 mmol) suspended in 10 mL dry diethyl ether, keeping the mixture continuously refluxing. In this way, reaction of all bromides with Mg was ensured. After addition, the mixture was stirred under reflux for 2 h, and then it was taken out via a syringe and added to the electrophile. The Grignard reagent was titrated according to known procedure.[23] The same procedure was used for preparation of a mixture of 25 Grignard reagents using 2 mmol of each bromide, 2.5 g Mg (0.1 mol) in 50 mL Et_2O.

General Procedure for Conjugate Addition of Grignard Reagents to (1*R*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester. (1*R*,5*S*)-8-Methyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic acid methyl ester (**2**, 0.7 mmol)[22] was dissolved in 10 mL dry diethyl ether, and the solution was cooled to −40 °C. The freshly prepared solution of Grignard reagent (1.4 mmol) was diluted with dry diethyl ether to a concentration of ∼0.4 M and added over 1.5 h using a syringe pump. After addition, the mixture was stirred at −40 °C for 2 h, then the reaction mixture was cooled to −78 °C and quenched by adding TFA (1.4 mmol) in 1 mL of dry diethyl ether. The mixture was stirred for 10 min at −78 °C before it was allowed to warm to 0 °C. A 10-mL portion of water and 10 mL diethyl ether were added, and the aqueous phase was acidified to pH 1 using a 2 M HCl solution. After extraction, the organic phase was discarded, and NH_4OH was added to the aqueous phase until pH 10. The aqueous phase was extracted with diethyl ether (5 × 10 mL), and the combined organic phases were washed with 10 mL of water, followed by 10 mL of an aqueous saturated solution of NaCl. Before concentration in vacuo, the organic phase was dried over $MgSO_4$. Further purification by column chromatography was only done for individual compounds. The same procedure was used for conjugate addition to (1*R*,5*S*)-8-alkyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic acid methyl esters **9**−**13**.

(1*R*,2*S*,3*S*,5*S*)-3-(3,4-Dimethylphenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (4). Flash chromatography: 30/10/1 pentane/Et_2O/Et_3N; R_f = 0.24; yield, 40%; $[\alpha]_D^{20} = -41°$ (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04−7.00 (m, 3H), 3.55 (m, 1H), 3.51 (s, 3H), 3.37−3.33 (m, 1H), 2.95 (dt, 1H, J = 4.8 Hz, J = 12.8 Hz), 2.91−2.88 (m, 1H), 2.56 (dt, 1H, J = 2.8 Hz, J = 12.8 Hz), 2.22 (s, 6H), 2.20 (s, 3H), 2.20−2.04 (m, 2H), 1.75−1.56 (m, 3H). ^{13}C NMR (100 MHz, CDCl3): δ 172.3, 140.5, 135.9, 133.9, 129.3, 128.7, 124.7, 65.4, 62.4, 52.8, 51.1, 42.1, 34.3, 33.3, 26.0, 25.3, 20.0, 19.3. HRMS(ES): *m*/*z* calcd for $C_{18}H_{25}NO_2$ + H, 288.1963; found, 288.1957.

(1*R*,2*S*,3*S*,5*S*)-3-Cyclohexyl-8-methyl-8-azabicyclo[3.2.1]-octane-2-carboxylic Acid Methyl Ester (6). Chromatography: 30/10/1 pentane/Et_2O/Et_3N; R_f = 0.29 yield, 36%; $[\alpha]_D^{20} = -9°$ (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.63 (s, 3H), 3.39 (m, 1H), 3.17−3.12 (m, 1H), 2.49 (t, 1H, J = 4.0 Hz), 2.11 (s, 3H), 2.06−188 (m, 2H), 1.82 (br.d, 1H, J = 12.8 Hz), 1.75 (dt, 1H, J = 2.8 Hz, J = 12.8 Hz), 1.66−1.36 (m, 8H), 1.23−0.99 (m, 4H), 0.68 (d quintet, 2H, J = 3.0, J = 12 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 173.3, 65.4, 62.6, 51.2, 48.6, 42.0, 37.9, 35.4, 34.6, 31.6, 31.0, 26.7, 26.2, 26.1, 25.5, 25.3. HRMS(ES): *m*/*z* calcd for $C_{16}H_{27}NO_2$ + H, 266.2120; found, 266.2120.

(1*R*,2*S*,3*S*,5*S*)-3-(3-Methylphenyl)-8-methyl-8-azabicyclo-[3.2.1]octane-2-carboxylic Acid Methyl Ester (5). Flash-chromatography: 30/10/1 pentane/Et_2O/Et_3N; R_f = 0.21; yield, 61%; $[\alpha]_D^{20} = -44°$ (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15 (t, 1H, J = 7.6 Hz), 7.08−7.03 (m, 2H), 6.96 (d, 1H, J = 6.8 Hz), 3.55 (dd, 1H, J = 2.8 Hz, J = 7.0 Hz), 3.49 (s, 3H), 3.38−3.33 (m, 1H), 2.97 (dt, 1H, J = 5.2 Hz, J = 12.8 Hz), 2.93−2.89 (m, 1H), 2.57 (dt, 1H, J = 2.8 Hz, J = 12.8 Hz), 2.31 (s, 3H), 2.23 (s, 3H), 2.21−2.15 (m, 1H), 2.14−2.02 (m, 1H), 1.75−1.65 (m, 2H), 1.60 (ddd, 1H, J = 3.7 Hz, J = 9.2 Hz, J = 13.0 Hz). ^{13}C NMR (100 MHz, CDCl3): δ 172.0, 142.9, 137.1, 128.0, 127.7, 126.4, 124.2, 65.2, 62.2, 52.7, 50.9, 41.9, 34.0, 33.5, 25.8, 25.1, 21.4. HRMS(ES): *m*/*z* calcd for $C_{17}H_{23}NO_2$ + H, 274.1807; found, 274.1803.

(1*R*,2*S*,3*S*,5*S*)-3-(4-*tert*-Butylphenyl)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (7).[24] Flash-chromatography: 30/10/1 pentane/Et_2O/Et_3N; R_f = 0.24; yield, 54%; $[\alpha]_D^{20} = -43°$ (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29 (d, 2H, J = 8 Hz), 7.19 (d, 2H, J = 8 Hz), 7.09 (dd, 1H, J = 2.8 Hz, J = 6.4 Hz), 3.50 (s, 3H), 3.38−3.34 (m, 1H), 2.98 (dt, 1H, J = 5.2 Hz, J = 12.8 Hz), 3.66−3.56 (m, 1H), 2.231 (s, 3H), 2.22−2.14 (m, 1H), 2.13−2.03 (m, 1H), 1.77−1.55 (m, 4H), 1.29 (s, 9H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 148.5, 139.9, 127.1, 124.9, 65.5, 62.4, 52.8, 51.1, 42.1, 34.3, 34.2, 33.5, 31.4, 26.0, 25.3. HRMS(ES): *m*/*z* calcd for $C_{20}H_{29}NO_2$ + H: 316.2276; found, 316.2276.

(1*R*,2*S*,3*S*,5*S*)-3-Butyl-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (3). Flash-chromatography: 30/10/1 pentane/Et_2O/Et_3N; R_f = 0.19; yield, 76%; $[\alpha]_D^{20} = -22°$ (*c* 1; MeOH); ^{1}H NMR (200 MHz, $CDCl_3$): δ 3.60 (s, 3H), 3.41−3.30 (m, 1H), 3.15−3.05 (m, 1H), 2.36−2.34 (m, 1H), 2.09 (s, 3H), 2.05−1.87 (m, 2H) 1.79 (dt, 1H, J = 2.8 Hz, J = 12 Hz), 1.57−1.04 (m, 10H), 0.79 (t, 3H, J = 7 Hz). ^{13}C NMR (50 MHz, $CDCl_3$): δ 173.0, 65.5, 62.5, 51.1, 50.3, 42.0, 36.8, 32.2, 29.9, 29.5, 25.6, 25.4, 22.9, 14.2. HRMS(ES): *m*/*z* calcd for $C_{14}H_{25}NO_2$ + H, 240.1963; found, 240.1961.

(1*R*,5*S*)-8-Azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (8). To a solution of methyl ecgonidine (**2**) (2.6 g; 14.4 mmol) in 80 mL of dichloroethane were added Na_2CO_3 (6.5 g; 61 mmol) and 1-chloroethyl chloroformate (7.8 mL; 72 mmol). The mixture was allowed to for 5 h. After cooling, the mixture was filtered, and the volatiles were evaporated. The resulting syrup was redissolved in 50 mL of MeOH and stirred overnight at room temperature. After evaporation, the product was purified by column chromatography (9:1 dichloromethane/MeOH + 1% NH_4OH, R_f = 0.4), and 1.65 g of a yellow syrup was isolated (75% based

on recovered starting material). ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.72−6.70 (m, 1H), 4.14 (m, 1H), 3.73 (s, 3H), 3.69 (t, 1H, J = 5.2 Hz), 2.618 (br d, 1H, J = 19.6 Hz), 2.08−1.90 (m, 4H), 1.67 (br s, 1H), 1.58−1.50 (m, 1H).

General Procedure for N-Alkylation on (1*R*,5*S*)-8-Azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (9−13). To a solution of 250 mg of **8** in 10 mL of MeCN were added 2 g K_2CO_3 (14 mmol) and 275 mg KI (1.7 mmol). One (1.65 mmol) or five different alkyl bromides in equimolar amounts (0.33 mmol of each) were added to the solution, which was left refluxing overnight (18 h). After cooling to room temperature, filtration, and evaporation, 10 mL of water and 10 mL of diethyl ether were added, and the aqueous phase was acidified to pH 1 using a 2 M HCl solution. After extraction, the organic phase was discarded, and NH_4OH was added to the aqueous phase until pH 10. The aqueous phase was extracted with diethyl ether (5 × 10 mL), and the combined organic phases were washed with 10 mL of water, followed by 10 mL of an aqueous saturated solution of NaCl. Before concentration in vacuo, the organic phase was dried over $MgSO_4$. Purification was done on silica gel.

(1*R*,5*S*)-8-Ethyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (9). Flash-chromatography: 19/1 dichloromethane/MeOH + 1% NH_4OH; R_f = 0.31; yield, 72%; $[\alpha]_D^{20}$ = −40.2° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82−6.80 (m, 1H), 3.90 (d, 1H, J = 5.6 Hz), 3.72 (s, 3H), 3.35 (t, 1H, J = 5.6 Hz), 2.58 (br d, 1H, J = 20 Hz), 2.49 (dq, 2H, J = 1.6 Hz, J = 7.2 Hz), 2.19−2.03 (m, 2H), 1.85−1.77 (m, 2H), 1.53−1.45 (m, 1H), 1.11 (t, 3H, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 136.5, 134.3, 56.2, 54.9, 51.8, 42.8, 34.4, 31.9, 30.1, 14.0. HRMS(ES): *m*/*z* calcd for $C_{11}H_{17}NO_2$ + H, 196.1338; found, 196.1338.

(1*R*,5*S*)-8-Propyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (10). Flash-chromatography: 19/1 dichloromethane/MeOH + 1% NH_4OH; R_f = 0.34; yield, 80%; $[\alpha]_D^{20}$ = −38.4° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83−6.79 (m, 1H), 3.87 (d, 1H, J = 5.6 Hz), 3.72 (s, 3H), 3.32 (t, 1H, J = 4.8 Hz), 2.58 (br d, 1H, J = 19.6 Hz), 2.41−2.36 (m, 2H), 2.18−2.03 (m, 2H), 1.84−1.75 (m, 2H), 1.57−1.44 (m, 3H), 0.90 (t, 3H, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 136.5, 134.2, 56.4, 55.1, 51.7, 51.0, 34.4, 31.7, 30.1, 22.2, 12.3. HRMS(ES): *m*/*z* calcd for $C_{12}H_{19}NO_2$ + H, 210.1494; found, 210.1490.

(1*R*,5*S*)-8-Butyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (11). Flash-chromatography: 19/1 dichloromethane/MeOH + 1% NH_4OH; R_f = 0.36; yield, 86%; $[\alpha]_D^{20}$ = −36.2° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82−6.79 (m, 1H), 3.87 (d, 1H, J = 5.6 Hz), 3.72 (s, 3H), 3.32 (t, 1H, J = 5.6 Hz), 2.57 (br d, 1H, J = 19.6 Hz), 2.43−2.40 (m, 2H), 2.18−2.03 (m, 2H), 1.83−1.75 (m, 2H), 1.52−1.44 (m, 3H), 1.32 (sixtet, 2H, J = 7.6 Hz), 0.90 (t, 3H, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 136.5, 134.2, 56.5, 55.1, 51.7, 48.7, 34.4, 31.7, 31.2, 30.1, 21.0, 14.2. HRMS(ES): *m*/*z* calcd for $C_{13}H_{21}NO_2$ + H, 224.1651; found, 224.1658.

(1*R*,5*S*)-8-Pentyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (12). Flash-chromatography: 19/1 dichloromethane/MeOH + 1% NH_4OH; R_f = 0.39; yield, 78%; $[\alpha]_D^{20}$ = −34.7° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83−6.80 (m, 1H), 3.88 (d, 1H, J = 5.6 Hz), 3.73 (s, 3H), 3.33 (t, 1H, J = 5.6 Hz), 2.58 (br d, 1H, J = 19.6 Hz), 2.44−2.38 (m, 2H), 2.19−2.04 (m, 2H), 1.84−1.74 (m, 2H), 1.54−1.44 (m, 3H), 1.36−1.22 (m, 4H), 0.88 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.8, 136.5, 134.2, 56.5, 55.1, 51.7, 49.1, 34.4, 31.8, 30.1 (double intensity), 29.0, 22.8, 14.2. HRMS(ES): *m*/*z* calcd for $C_{14}H_{23}NO_2$ + H, 238.1807; found, 238.1799.

(1*R*,5*S*)-8-Hexyl-8-azabicyclo[3.2.1]oct-2-ene-2-carboxylic Acid Methyl Ester (13). Flash-chromatography: 19/1 dichloromethane/MeOH + 1% NH_4OH; R_f = 0.42; yield, 84%; $[\alpha]_D^{20}$ = −31.8° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83−6.80 (m, 1H), 3.89 (d, 1H, J = 5.6 Hz), 3.72 (s, 3H), 3.34 (t, 1H, J = 5.6 Hz), 2.59 (br d, 1H, J = 19.6 Hz), 2.45−2.39 (m, 2H), 2.20−2.04 (m, 2H), 1.85−1.76 (m, 2H), 1.54−1.44 (m, 3H), 1.34−1.23 (m, 6H), 0.87 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.7, 136.5, 134.1, 56.5, 55.2, 51.7, 49.0, 34.4, 31.9, 31.7, 30.0, 28.9, 27.5, 22.7, 14.2. HRMS(ES): *m*/*z* calcd for $C_{15}H_{25}NO_2$ + H, 252.1963; found, 252.1953.

(1*R*,2*S*,3*S*,5*S*)-3-(3,4-Dimethylphenyl)-8-ethyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (15). Flash-chromatography: 40/10/1 pentane/Et_2O/Et_3N; R_f = 0.2; yield, 47%; $[\alpha]_D^{20}$ = −51.0° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.05−7.01 (m, 3H), 3.76−3.71 (m, 1H), 3.52 (s, 3H), 3.44−3.39 (m, 1H), 2.98 (dt, 1H, J = 4.8 Hz, J = 12.8 Hz), 2.94−2.90 (m, 1H), 2.57 (dt, 1H, J = 2.8 Hz, J = 12.4 Hz), 2.40−2.25 (m, 2H), 2.23 (s, 3H), 2.20 (s, 3H), 2.13−1.94 (m, 2H), 1.75−1.57 (m, 3H), 1.00 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 140.7, 135.9, 133.8, 129.3, 128.9, 124.8, 62.0, 61.7, 52.9, 51.1, 47.3, 34.4, 34.0, 26.3, 25.8, 20.0, 19.4, 14.2. HRMS(ES): *m*/*z* calcd for $C_{19}H_{27}NO_2$ + H, 302.2120; found, 302.2126.

(1*R*,2*S*,3*S*,5*S*)-3-(3,4-Dimethylphenyl)-8-pentyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (14). Flash-chromatography: 60/10/1 pentane/Et_2O/Et_3N; R_f = 0.2; yield, 48%; $[\alpha]_D^{20}$ = −46.8° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.06−7.00 (m, 3H), 3.73−3.67 (m, 1H), 3.52 (s, 3H), 3.41−3.37 (m, 1H), 2.97 (dt, 1H, J = 4.8 Hz, J = 12.8 Hz), 2.94−2.90 (m, 1H), 2.59 (dt, 1H, J = 3.2 Hz, J = 12.4 Hz), 2.31−1.95 (m, 10H), 1.76−1.56 (m, 3H), 1.44−1.23 (m, 6H), 0.90 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.2, 140.8, 135.8, 133.8, 129.2, 128.8, 124.8, 62.8, 61.9, 53.7, 53.0, 50.9, 34.3, 33.9, 29.6, 28.9, 26.2, 25.9, 22.7, 20.0, 19.4, 14.2. HRMS(ES): *m*/*z* calcd for $C_{22}H_{33}NO_2$ + H, 344.2589; found, 344.2589.

(1*R*,2*S*,3*S*,5*S*)-3-(3,4-Dimethylphenyl)-8-hexyl-8-azabicyclo[3.2.1]octane-2-carboxylic Acid Methyl Ester (16). Flash-chromatography: 70/10/1 pentane/Et_2O/Et_3N; R_f = 0.2; yield, 40%; $[\alpha]_D^{20}$ = −50.4° (*c* 1; MeOH); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.04−7.0 (m, 3H), 3.71−3.66 (m, 1H), 3.50 (s, 3H), 3.40−3.35 (m, 1H), 3.00−2.87 (m, 2H), 2.57 (dt, 1H, J = 2.8 Hz, J = 12.4 Hz), 2.30−2.16 (m, 8H), 2.13−1.92 (m, 2H), 1.74−1.55 (m, 3H), 1.41−1.20 (m, 8H), 0.88 (t, 3H, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 172.3, 140.9, 135.8, 133.8, 129.3, 128.9, 124.8, 62.8, 61.9, 53.8, 53.0, 51.0, 34.4, 34.0, 32.0, 29.3, 27.1, 26.3, 26.0, 22.8,

20.0, 19.4, 14.2. HRMS(ES): *m/z* calcd for $C_{23}H_{35}NO_2$ + H, 358.2746; found, 358.2734.

Determination of K_i Values. 1. Cell Culture. Cell lines stably expressing hSERT, hDAT, or hNET were established by transfecting COS-1 cells with hSERT/hDAT/hNET inserted in the pIRES vector (BD Biosciences Clontech) also carrying a Blasticidin resistance gene. Cells were cultured in DMEM (BioWhitaker) supplemented with 10% FCS (Gibco Life Technologies), 1% penicillin/streptomycin (BioWhitaker), and 10 μg/mL of Blasticidin (Cayla) selection of transfected cells. After 14 days of selection, Blasticidin was adjusted to 2 μg/mL in the culture medium, and the cells were subcultured under this selection regime and grown at 37 °C, 5% CO_2, and 95% humidity.

2. Uptake Assay. For the uptake assay, stably transfected cells were seeded in 96-well microplates (Nunc) and grown at 37 °C, 5% CO_2, and 95% humidity for 2 days. Prior to the IC_{50} assay, the medium was aspirated, and the cells were washed in PBSCM (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, 0.1 mM $CaCl_2$, and 1 mM $MgCl_2$, pH 7.4) on an automatic microplate washer. The dilution series of the drug in PBSCM were supplemented with 30−100 nM tritiated 5-HT (Perkin-Elmer Life Sciences), or DA (Amersham Bisciences) was applied to the adhering cells to initiate uptake. Accumulation of 5-HT or DA was allowed to proceed for 10 min at 20 °C, and the assay was terminated by aspiration of the uptake media and washing with PBSCM. Scintillant (MicroScint 20 from Packard Bell) was used to solubilize cells, and accumulated radioactivity was quantifed on a Packard Topcounter. Concentration of the substrate was quantified by liquid scintillation counting on an Packard Tri-Carb.

3. Binding Assay. Membrane preparations for the binding assay were produced by scraping the stably transfected cells from cell culture dishes (Nunc), pelleting the cells in ice-cold PBSCM by centrifugation and homogenizing the cells in ice-cold Harvest buffer I (150 mM NaCl, 50 mM Tris, 20 mM EDTA) using an Ultra-Turrax (Janke & Kunkel AG) for 60 s. The membrane was pelleted by centrifugation at 12000*g* for 10 min at 4 °C and washed in ice-cold Harvest buffer I. The membranes were pelleted again and, finally, resuspended in PBSCM using the Ultraturrax briefly. Membrane preparations were aliquoted into 2-mL portions and stored at −80 °C until use. The concentration of total protein in the membrane preparation was determined with the MicroBCA kit (Pierce).

A concentration of 5 μg/well of membrane preparation was used with the chosen concentration of drug of interest in combination with 0.1−0.25 nM ^{125}I-RTI-55. Membrane and ligands were incubated for 1 h at 20 °C. Using a Filtermate cell harvester (Packard), membranes were captured on GF/B 96-well filterplates (Packard) presoaked with 0.5% polyethyleneimine (Merck) and washed thrice with ice-cold water. The filter in each well was dissolved in 40 μL Microscint 20 and scintillation counts were determined with a Packard Topcounter. Precise concentration of radioligand was quantified by liquid scintillation counting on a Packard Tri-Carb.

Data Analysis. Counts from the Packard Topcounter were fitted to a sigmoidal dose−response curve using the built-in nonlinear regression tool in the Graphpad Prism 3 software. From at least three independent experiments, the resulting IC_{50} values were transformed to K_i values using the equation described by Cheng and Prusoff.[25]

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Supporting Information Available. The contents of the Supporting Information include (1) Table 1 (K_i values of libraries I−V and a−e), (2) Table 2 (K_i values of libraries X_1−X_5, Y_1−Y_5, and Z_1−Z_5), (3) identification of compounds in libraries I−V and a−e (GC−MS and ESMS), (4) identification of 9−13 in mixture (GC−MS), and (5) ^{13}C NMR for new compounds **3**−**6** and **9**−**16**.

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