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A *de Novo* Design Probe of a Dopamine Receptor Ligand Based on a Theoretical Approach

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A trial to design *de novo* a dopamine (DA) receptor ligand was made, taking as the base four structural and electrostatic requirements: (1) a group simulating the interaction of the DA amino group with the TM3 aspartic acid of the receptor, (2) a group that can simulate the interaction of the DA *m*-hydroxyl group with the TM5 serine of the receptor, (3) a distance between these groups similar to that of the DA *anti*-coplanar conformer, and (4) a rigid structure keeping the distance between the groups right. After the design "on paper" of the models of four structures, quantum chemistry calculations were performed to check the properties of the molecules, and then the most encouraging ones were synthesized. None of the compounds synthesized was able to bind D_1- and D_2-dopamine receptor subtypes; this shows that the structural and electrostatic requirements considered in this work are insufficient. In particular, the presence of an arylethylamine moiety seems to be essential for the interaction of a ligand with the DA receptor.

INTRODUCTION

The neurotransmitter dopamine (DA) is involved in various central nervous system (CNS) disorders such as schizophrenia and Parkinson's disease. Structure–activity relationship studies on DA agonists and antagonists have attracted considerable interest during the last years. The results obtained have been used to discuss optimal features for DA receptor activation, and various hypothetical DA receptor models have been suggested. The same studies have given many lead compounds as well as apomorphine, aminodihydroxytetraline (ADTN), ergoline, and benzazepine derivatives (*1*).

Recently, in addition to the structure–activity studies, some attempts have been made to approach the problem in a more rational way, based on understanding the molecular mechanism of neurotransmitter–receptor interaction (*2*). This approach has been possible since the three-dimensional structure of DA receptors has been postulated by means of molecular modeling simulations.

Part of those studies concerned conformational analysis and molecular electro-

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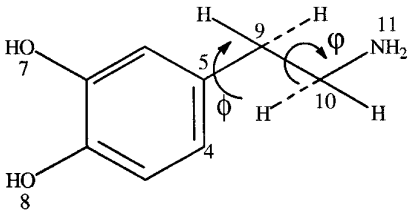


ϕ ≡ C4-C5-C9-C10 = 0.0°

φ ≡ C5-C9-C10-N11 = 180.0°

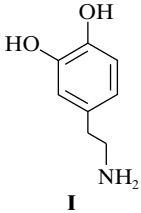
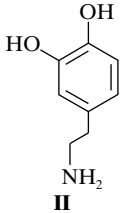
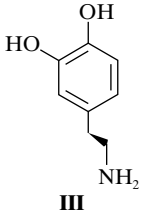
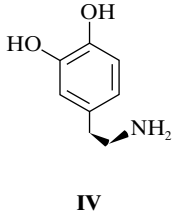
FIGURE 1

static potential distribution of DA (*3–6*). Theoretical conformational analysis performed for isolated and solvated DA molecule has revealed that its side chain may attain various conformations. The energy differences between them are small, but the *anti*-conformer with the catechol ring coplanar with the ethylamine (C–C–N) side chain (Fig. 1) is not energetically favored. This is not in agreement with experimental studies on conformationally restricted analogs which suggest that the *anti*-coplanar conformer is biologically active (*7*). Therefore, Edvardsen and Dahl (*5*) have proposed a "zipper" mechanism for the binding of flexible DA to its receptor, a suggestion based on molecular dynamics simulations. Molecular electrostatic potential calculations have revealed that the potential and charge distribution is very dependent on a conformation that can be important for DA-receptor recognition and DA agonist/antagonist selectivity.

Theoretical molecular modeling calculations have been performed for isolated DA receptor as well as for receptor–DA interacting structure (*8–12*). These studies have been possible since (1) the genes coding for different DA receptors, belonging to the large G-protein-linked family of receptors, have recently been isolated, cloned, and decoded (*13*); (2) the structure of bacteriorhodopsin—a structural analog of the G-protein-coupled receptors (GPCR)—has been resolved by cryomicroscopy study (*14*). The structural molecular modeling simulations, by homology to bacteriorhodopsin, together with hydropathicity analysis have revealed that the DA D_2 receptor (similar to other G-protein-coupled receptors) has seven α-helical transmembrane segments that form the central core with a putative ligand-binding site. It has also been found that defined amino acids can be responsible for DA–receptor interaction. The point mutations of DA receptors also support these data (*15*). Generally, it has been postulated that (1) the aspartic acidic residue on the third transmembrane domain (TM3) interacts with the protonated amino group of DA, (2) two serine residues on the fifth transmembrane domain (TM5) interact with the hydroxyl groups of DA, and (3) the aromatic nuclei of a few amino acids on TM3 and TM6 form the hydrophobic surrounding of DA, which is probably rearranged during the binding process.

Taking into acocunt the essential aspects of the molecular model of receptor–DA interaction and the results of DA structural studies, we attempted to design *de novo* a DA-receptor ligand. The design was based on four structural and electrostatic requirements: (1) a group simulating the interaction of the DA amino group with the TM3 aspartic acid of the receptor, (2) a group which can simulate the interaction

TABLE 1
Results of AM1 Calculations for DA Molecule[a]

Conformers	 **I**	 **II**	 **III**	 **IV**
	Torsional angle (degrees)			
N11–C10–C9–C5	178	−176	−177	73
C10–C9–C5–C4	−2	167	−100	−95
	Distance of atoms (Å)			
O7–N11	7.311	6.474	6.700	6.249
O8–N11	7.903	7.868	7.789	6.610
	Net atomic charge (a.u.)			
O7	−0.249	−0.250	−0.249	−0.252
O8	−0.271	−0.272	−0.271	−0.272
N11	−0.350	−0.342	−0.352	−0.348
	Energy (heat of formation) and relative energy (kcal/mol)			
E	−74.7	−74.9	−75.7	−74.6
ΔE	1.0	0.8	0.0	1.1
	Proton affinity of nitrogen atom N11 (kcal/mol)			
PA	216.2	216.2	216.2	216.2

[a] The numbering of atoms is presented in Fig. 1.

of the DA *m*-hydroxyl group with the TM5 serine of the receptor, (3) the distance between these groups should be similar, as for the *anti*-coplanar conformer **I** of DA (Table 1), and (4) a rigid structure keeping the distance between the groups right.

In accordance with those requirements the models of four structures were designed ''on paper'' (Fig. 2). Later quantum chemistry calculations were performed to check the properties of the molecules, and then the most encouraging ones were synthesized.

All well-known DA receptor ligands include in their structure an aromatic moiety. The aromatic ring is considered very important for ligand–receptor binding because it seems to interact with the aromatic nuclei of two phenylalanine residues on the DA receptor (*8*). However, the DA aromatic ring may also be seen as a flat structure able to penetrate into the receptor pocket surrounded by the aromatic moieties of the phenylalanine residues. On the other hand, studies developed in the field of β-adrenergic receptor (G-protein-coupled receptor) show that completely aliphatic molecules maintain β-blocking properties as well as the relative aromatic compounds (*16*). Moreover, we have not found any reference to negative biological test results being obtained by studying nonaromatic compounds as DA receptor ligands. Therefore, we decided to replace the aromatic ring of DA with a system of conjugated double bonds to obtain a planar structure. In addition, since a group

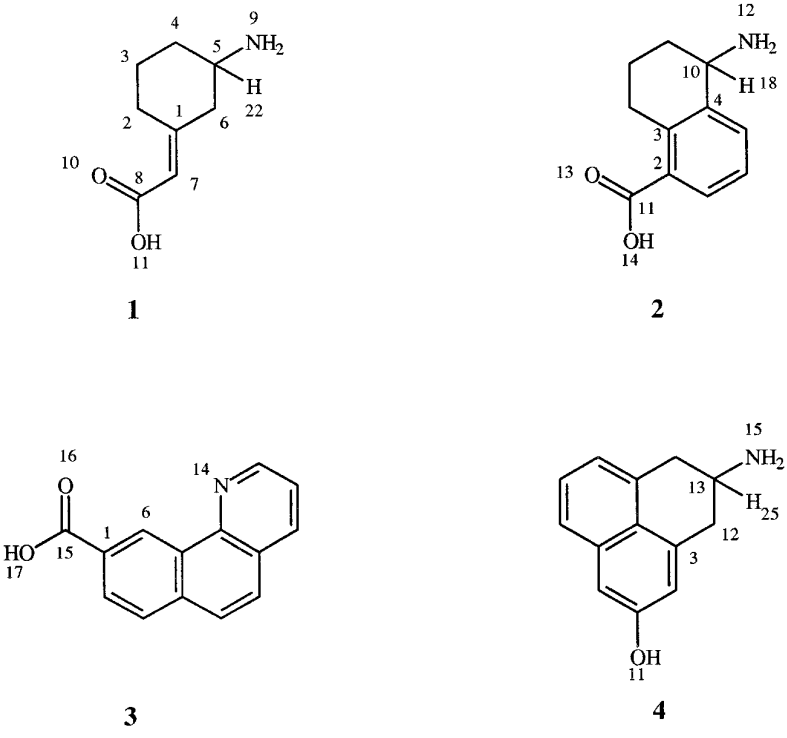


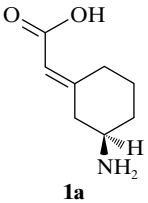
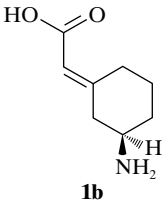
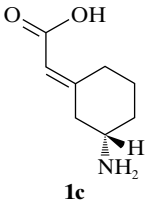
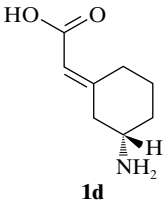
FIG. 2. The atoms were automatically numbered by the Chem3D program.

simulating the phenolic hydroxyl connected to the planar aromatic ring and able to interact with the serine residue on TM5 is necessary, we have chosen the α–β unsaturated carboxylic acid moiety. On the basis of this, we designed structure **1.** Compound **2** was seen as a more complex α–β unsaturated carboxylic acid which contains molecule **1** embedded in a more rigid framework. Moreover, the carboxyl group on compounds **1, 2,** and **3** can interact with the serine residue on TM5 as hydrogen bond donor or acceptor. Structure **4,** like DA, bears a phenolic group. Models **1, 2,** and **4** have an amino group very similar to that of DA, and compound **3** contains a nitrogen atom which can simulate the DA amino group.

RESULTS AND DISCUSSION

The quantum chemistry calculations for the four DA conformers shown in Table 1 were performed. Two of them, **I** and **II,** are *anti*-coplanar conformers. The *anti*-perpendicular conformer (**III**) is found in the crystal state and the *gauche*-perpendicular one (**IV**) is the most stable conformer found by Edvardsen and Dahl (*5*) in molecular dynamics simulations for protonated DA.

TABLE 2
Results of AM1 Calculations for Compound **1** (*E* Isomer)[a]

Conformers	 **1a**	 **1b**	 **1c**	 **1d**
		Torsional angle (degrees)		
N9–C5–C4–C3	−73	−73	179	−178
H22–C5–C4–C3	171	171	−59	−56
O10–C8–C7–C1	−148	0	−149	5
O11–C8–C7–C1	−33	180	33	−176
		Distance of atoms (Å)		
O10–N9	5.718	5.636	7.054	6.796
O11–N9	5.800	5.899	6.748	7.068
		Net atomic charge (a.u.)		
O10	−0.366	−0.375	−0.367	−0.377
O11	−0.319	−0.319	−0.320	−0.319
N9	−0.336	−0.336	−0.347	−0.347
		Energy (heat of formation) and relative energy (kcal/mol)		
E	−101.4	−102.7	−99.5	−100.9
Δ*E*	1.3	0.0	3.2	1.8
		Proteon affinity of nitrogen atom N9 (kcal/mol)		
PA	215.1	215.1	215.1	215.1

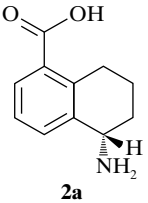
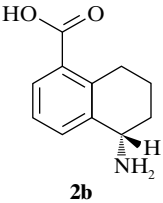
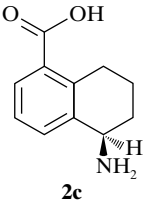
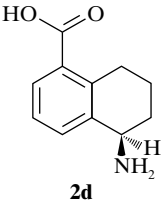
[a] The numbering of atoms is presented in Fig. 2.

In our study, conformer **III** was found to have the lowest energy (heat of formation). This result differs from the studies of Urban *et al.* (*3*), who found that the *gauche*-perpendicular conformer (our **IV**) is the most stable one for neutral DA, but at the same time they did not give energy values for the *anti*-perpendicular conformer (our **III**), so it is difficult to discuss the discrepancy.

It was also found, within our calculations, that the *anti*-coplanar conformers **I** and **II** lie only approximately 1.0 kcal/mol above conformer **III.** Generally, since the differences of energy between conformers are not very big, and conformer **I** is postulated as the active one (*7*), we chose it as the reference for other studied molecules.

The results of calculations performed for compounds **1–4** are presented in Tables 2–5. The carbon atoms C5 of **1** and C10 of **2** (Fig. 2) can have different configurations with regard to the position of the amino group. These possibilities were accounted for in the calculations (Tables 2 and 3). The energy difference between the conformers of the same compound sometimes assumes a value of several kilocalories per mole (especially for compounds **2** and **4**). However, it is difficult to analyze this, since the conformer populations at the receptor site can be different from those in

TABLE 3
Results of AM1 Calculations for Compound **2**[a]

Conformers	 **2a**	 **2b**	 **2c**	 **2d**
		Torsional angle (degrees)		
N12–C10–C4–C3	−110	−110	143	146
H18–C10–C4–C3	135	135	−97	−93
O13–C11–C2–C3	160	−10	159	−12
O14–C11–C2–C3	−21	171	−22	168
		Distance of atoms (Å)		
O13–N12	6.939	6.332	7.255	6.623
O14–N12	6.272	6.952	6.447	7.227
		Net atomic charge (a.u.)		
O13	−0.370	−0.373	−0.371	−0.373
O14	−0.316	−0.320	−0.315	−0.320
N12	−0.334	−0.334	−0.345	−0.349
		Energy (heat of formation) and relative energy (kcal/mol)		
E	−81.1	−82.0	−77.4	−78.6
ΔE	0.9	0.9	4.6	3.4
		Proteon affinity of nitrogen atom N12 (kcal/mol)		
PA	218.4	218.4	218.4	218.4

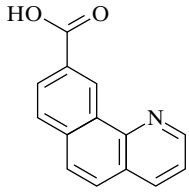
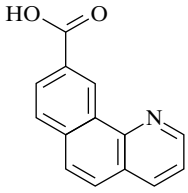
[a] The numbering of atoms is presented in Fig. 2.

the gas phase. It also means that the low energetic conformer in the gas phase need not be the active one. The same problem regards the DA molecule.

When the distances between functional groups (or rather atoms) are considered, compound **1** (conformers **1c** and **1d**), compound **2** (conformers **2c** and **2d** and, to some extent, **2a** and **2b**), and compound **4** (conformer **4a**) are the best simulators of the DA molecule. The values of net atomic charge for oxygen atoms in the compounds with carboxyl groups (**1–3**) are obviously more negative than for those in DA, so stronger electrostatic interaction of these groups with the serine residue on the DA receptor can be expected. The values of net atomic charge for the nitrogen atom are very similar for all compounds (except for compound **3**), and comparable with those for the DA molecule. The value for proton affinity of the nitrogen atom, which can be used to estimate to some extent the possibility of forming a hydrogen bond, is better for compounds **1–3** than for the DA molecule. The amino group (i.e., the nitrogen atom) in these compounds can form a similar or stronger ionic bond with aspartic acid residue on the DA receptor.

Taking into account the distance requirement, which may be very important for a possible interaction of the functional groups with the DA receptor, compound **3** (Fig. 2, Table 4) was excluded from further studies. The results of molecular modeling calculations for the other structures **1, 2,** and **4** were encouraging, so it was

TABLE 4
Results of AM1 Calculations for Compound **3**[a]

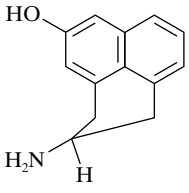
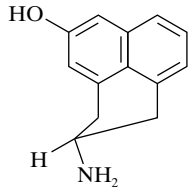
Conformers	 **3a**	 **3b**
	Torsional angle (degrees)	
O16–C15–C1–C6	0	180
O17–C15–C1–C6	180	0
	Distance of atoms (Å)	
O16–N14	5.157	6.432
O17–N14	6.468	4.954
	Net atomic charge (a.u.)	
O16	−0.361	−0.368
O17	−0.319	−0.313
N14	−0.137	−0.138
	Energy (heat of formation) and relative energy (kcal/mol)	
E	−21.2	−21.3
ΔE	0.1	0.0
	Proton affinity of nitrogen atom N14 (kcal/mol)	
PA	218.2	218.2

[a] The numbering of atoms is presented in Fig. 2.

decided to synthesize these compounds. Meanwhile, however, we had found some reports (*17*) about dopaminergic activity of 2-amino-5-hydroxyphenalene **4** derivatives, and thus only compounds **1** and **2** were synthesized. Since the hydroxyl of the carboxylic acid group is more acidic than phenolic hydroxyl, we also synthesized the ester and amide derivatives to evaluate the influence of acidity on the ligand–receptor interaction.

The synthetic approach to 3-aminocyclohexylidene acetic acid **1** (Scheme 1) started from commercially available 2-cyclohexen-1-one, which was reacted with azidotrimethylsilane and ethylene glycol to give 3-azidocyclohexanone ethylene ketal **5.** This compound was reduced by lithium aluminum hydride to the 3-aminocyclohexanone ethylene ketal **6,** which was treated with acetic anhydride to give acetamide **7.** After ketal cleavage, 3-acetamidocyclohexanone **8** was reacted with triethylphosphonacetate to obtain the 3-*N*-acetylaminocyclohexylidenacetic acid ethyl ester **9** as *E*–*Z* isomers mixture. Single isomers were separated by recrystallization; the exact structures were identified by NMR analysis observing the NOE (nuclear Overhouser effect) between the hydrogen on C7 and those on C6 (*E* isomer) (numbering in Fig. 2) or on C2 (*Z* isomer). *E* isomer **9** was hydrolyzed to target compound **1.** Then the amino group was protected with a buthyloxycarbonyl (Boc) group. The *N*-Boc-amino acid **10** was treated with ethyl chloroformate, triethylamine and ammonia to give the corresponding amide **11,** or with potassium

TABLE 5
Results of AM1 Calculations for Compound **4**[a]

Conformers	 **4a**	 **4b**
	Torsional angle (degrees)	
O15–C13–C12–C3	176	70
H25–C13–C12–C3	60	−169
	Distance of atoms (Å)	
O11–N15	7.314	6.287
	Net atomic charge (a.u.)	
O11	−0.252	−0.254
N15	−0.335	−0.343
	Energy (heat of formation) and relative energy (kcal/mol)	
E	−13.6	−10.0
	Proton affinity of nitrogen atom N15 (kcal/mol)	
PA	212.9	212.9

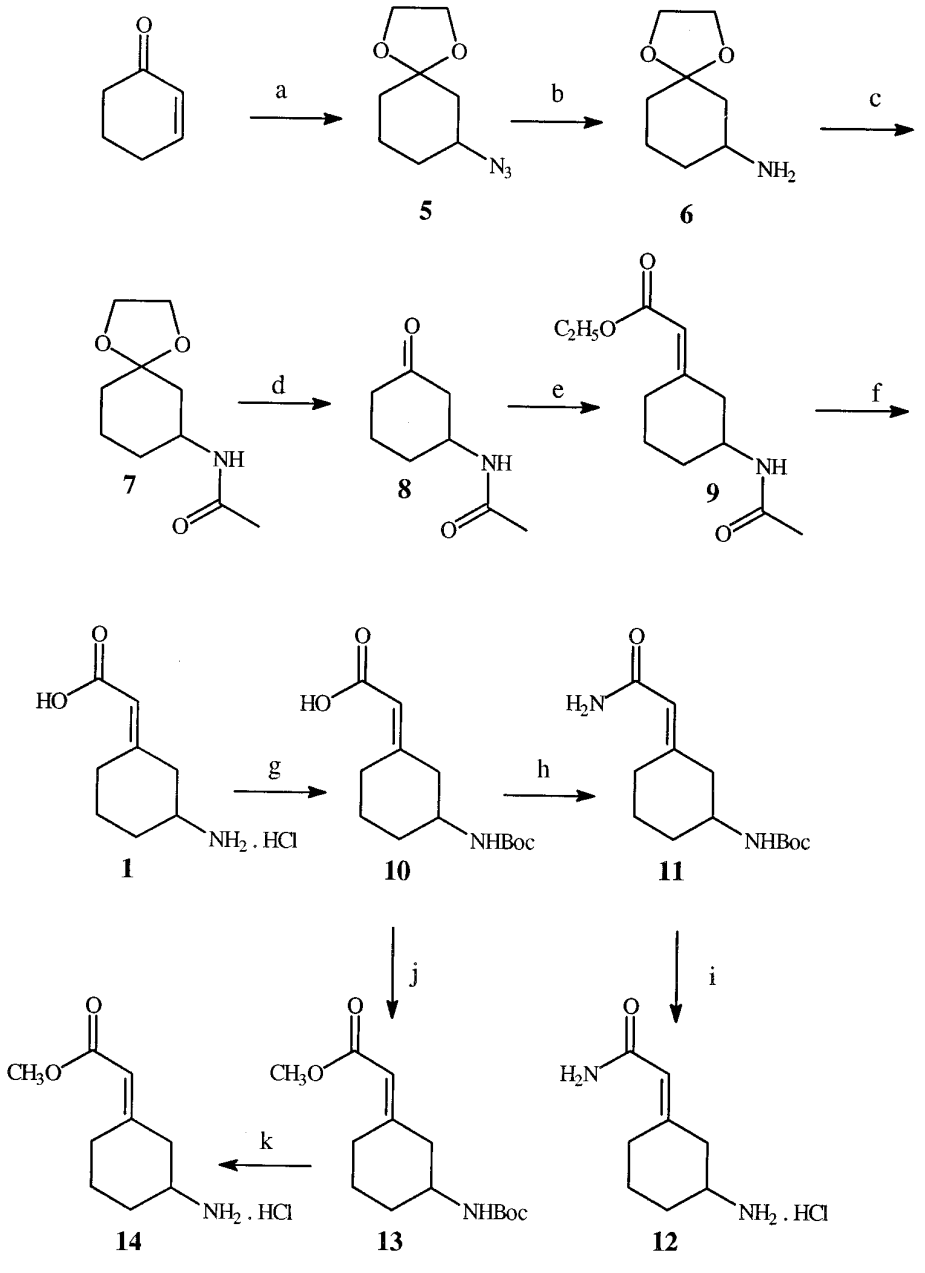
[a] The numbering of atoms is presented in Fig. 2.

carbonate and iodomethane to give ester **13.** The Boc group cleavage gave, respectively, the 3-aminocyclohexylidenacetamide **12** and the 3-aminocyclohexylidenacetic acid methyl ester **14.**

Scheme 2 shows the synthetic approach to 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **2.** 5-Oxo-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid **15** (*18–20*) was reacted with hydroxylamine to give the corresponding oxime **16.** This was hydrogenated over Pd/C in trifluoroacetic acid to amine **2.** The amino group was protected with a Boc group, and the *N*-Boc-amino acid **19** was treated with *N*-hydroxysuccinimide (NHS), dicyclohexylcarbodiimide (DCC), and ammonia to give the corresponding amide **20.** The Boc group cleavage gave the 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxamide **21.** Treatment of amino acid **2** with thionyl chloride and methanol gave the 5-amino-5,6,7,8-tetrahydro-1-naphthalenecarboxylic acid ethyl ester **18.**

To evaluate the affinities for D_1 and D_2 DA receptors, compounds **1, 2, 12, 14, 18,** and **21** and the reference compound were tested in *in vitro* radioligand competition assays on rat striatal membrane using [^{3}H]SCH23390 and [^{3}H]spiperone as radioligands Table 6.

Although some molecular parameters calculated by theoretical methods for compounds **1** and **2** agree with the reference DA conformer **I** (claimed to be active), no compounds show any significant affinity to the DA receptor. These results suggest that an aromatic ring seems to be necessary for the ligand–receptor interaction. Moreover, the α–β unsaturated carboxylic moiety cannot be considered a bioisost-



SCHEME. 1. (a) $(CH_3)_3SiN_3$, $SiCl_4$, $HOCH_2CH_2OH$; (b) $LiAlH_4$; (c) $(CH_3CO)_2O$; (d) CH_3COOH; (e) $(C_2H_5O)_3POCH_2COOC_2H_5$, NaH; (f) 2 N NaOH, 6 N HCl; (g) (t-BuOCO)$_2$O, $(C_2H_5)_3N$; (h) $ClCOOC_2H_5$, $(C_2H_5)_3N$, NH_3; (i) HCl; (j) CH_3I, K_2CO_3; (k) HCl.