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Silicon-Mediated Inactivation of Diamine Oxidase

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The design, synthesis, and biologic evaluation of potential silicon-containing inhibitors of diamine oxidases (siladiaminopropane **1,** silaputrescine **2,** and sila analogs of cadaverine **3, 4,** and **5**) are described. All compounds have been prepared independently. The common feature in the reported syntheses is the way chosen to introduce the amine group relative to silicon: the Gabriel-type approach to obtaining aminomethyl- and aminopropylsilanes and the Mitsunobu-type approach to obtaining aminoethylsilanes. Among the synthesized silane diamines, compounds **1** and **3** have been found to be time-dependent inhibitors of diamine oxidases prepared from hog kidney and rat small intestine. These results extend and generalize the concept of silicon-based inactivation of amine oxidases.

INTRODUCTION

It was recently reported that (aminoalkyl)trimethylsilanes and derivatives produce mechanism-based inactivation of the flavin-containing monoamine oxidases (*1–4*); of the copper-containing bovine plasma amine oxidase (*5*), for which recent studies have shown that the active-site cofactor is 2,4,5-trihydroxyphenylalanine (TOPA) (*6–8*); and of semicarbazide-sensitive amine oxidase (*9*), an enzyme insensitive to copper-chelating agents (*10*) and for which the cofactor (presumably containing a carbonyl group) is unknown (*11*). With the exception of monoamine oxidase (MAO, EC 1.4.3.4), the aforementioned oxidases belong to the same class of enzymes (EC 1.4.3.6) according to the Nomenclature Committee of the International Union of Biochemistry and Molecular Biology (*12*) and are inhibited by aminoguanidine, semicarbazide, or other hydrazines. However, the physiological significance of these enzymes is unclear (*13*). This is due in large part to the unavailability of selective inhibitors of these enzymes. We thus report our own work on the design, synthesis, and biological evaluation of new potential irreversible silicon-based inhibitors of diamine oxidase.

From the mechanism of copper-dependent amine oxidases (*8*),

$$E_{ox} + H_3\overset{+}{N}-CH_2R \rightleftharpoons E_{red}-H\overset{+}{N}=CHR,$$

$$E_{red}-H\overset{+}{N}=CHR + H_2O \rightarrow E_{red}-NH_2 + O=CHR,$$

$$E_{red}-NH_2 + O_2 \rightarrow E_{ox} + H_2O_2 + H_4\overset{+}{N}$$

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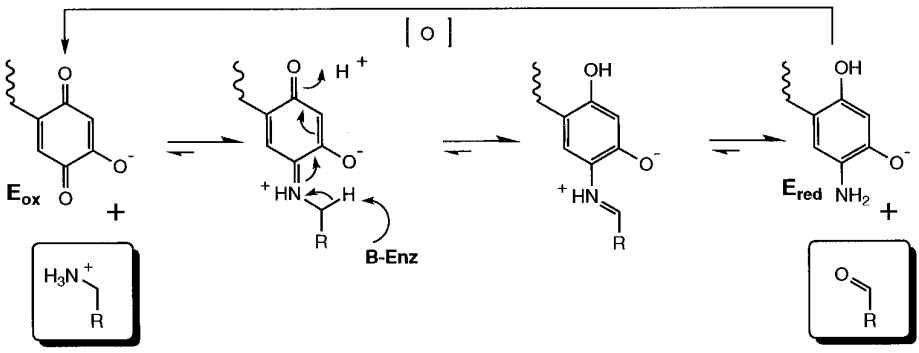


SCHEME 1. Proposed mechanism of oxidation of an amine by a TOPA-bound diamine oxidase.

(see Scheme 1), we expected that processing of the following silane diamines **1–5** (Fig. 1) by diamine oxidase might result formally in transaminative desilylation and attachment of $[R(CH_3)_2Si]$ to a nucleophilic residue of the active site (Nu−E; Schemes 2 and 3, path a) or in simple transamination (Schemes 2 and 3, path b) which would produce $E(TOPA)H\overset{+}{N}{=}CHSi(CH_3)_2R$ or $E(TOPA)H\overset{+}{N}{=}CHCH_2Si(CH_3)_2R$, potential alkylating or silylated agents, respectively (*1*, *3*).

Several analogs of putrescine, the prototype substrate of diamine oxidase (*14*), have been prepared. Thus, siladiaminopropane **1,** silaputrescine **2,** and sila analogs of cadaverine **3, 4,** and **5** were synthesized (Fig. 1) and tested for their potential inhibitor activity on various amine oxidases.

RESULTS AND DISCUSSION

Chemistry

All compounds were synthesized independently. Two general synthetic approaches were used: When the protected amine was *one* or *three* methylene units away from the silicon atom, structures of type A or C (Fig. 2) were obtained through

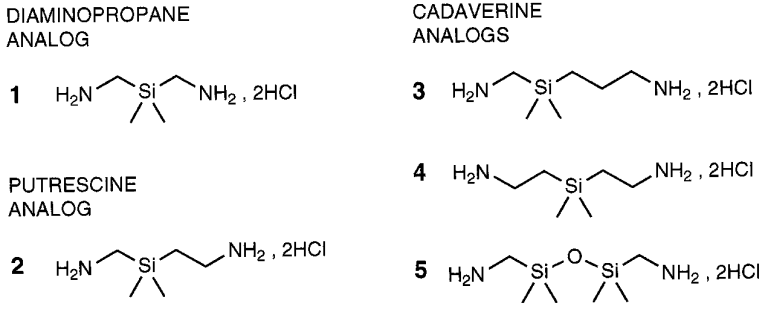
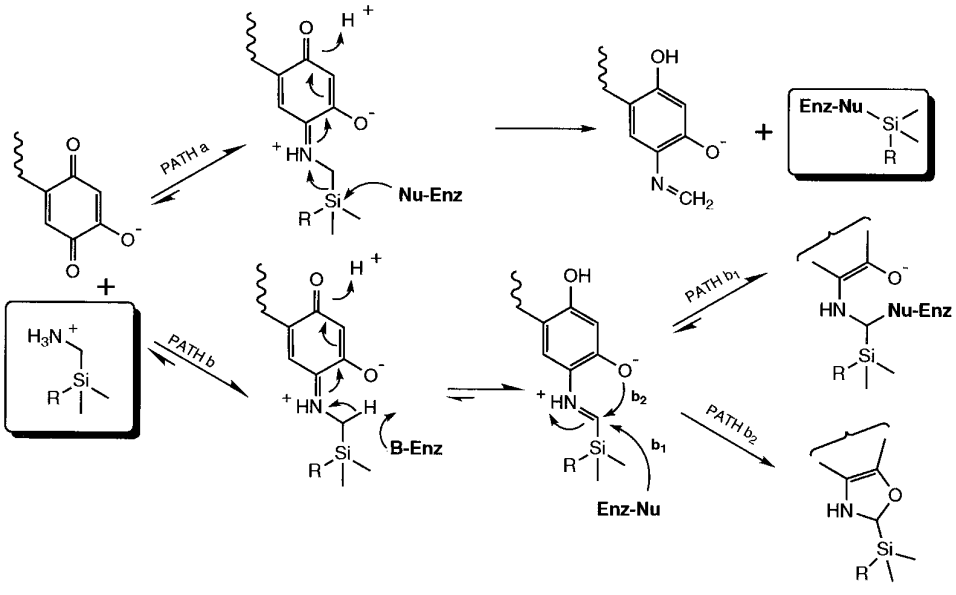
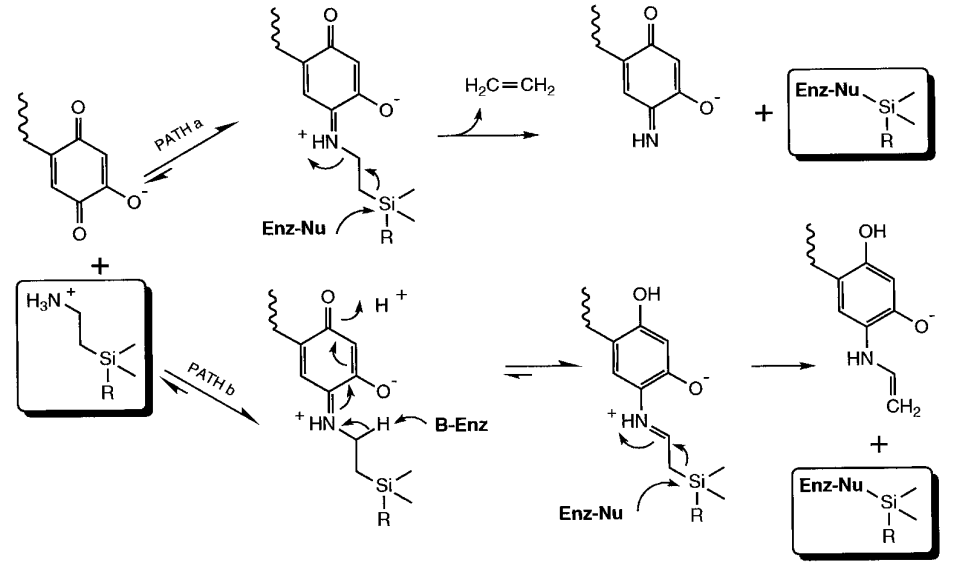


FIGURE 1



SCHEME 2. Possible mechanism of inactivation of a TOPA-bound diamine oxidase by silicon methylamines.



SCHEME 3. Possible mechanism of inactivation of a TOPA-bound diamine oxidase by silicon ethylamines.

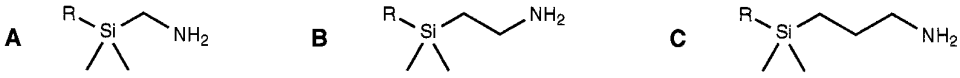


FIGURE 2

straightforward substitution of the proper α-halogenoalkylsilane with potassium phthalimide, sodium azide, or di-*tert*-butyliminodicarboxylate, potassium salt (*15*); however, when the protected amine was *two* methylene units away from the silicon atom, structures of type B (Fig. 2) were prepared through a Mitsunobu-type approach (for review, see *16*) from the proper hydroxyl precursor. Mild neutral conditions were necessary in this case to avoid the well-known β-elimination reaction of β-functional organosilanes (for a review of this principle, see *17*).

The last step in the described syntheses was an acidic cleavage of the properly protected diamines, removal under basic conditions affording the desired diamine in very low yields (see synthesis of **2**).

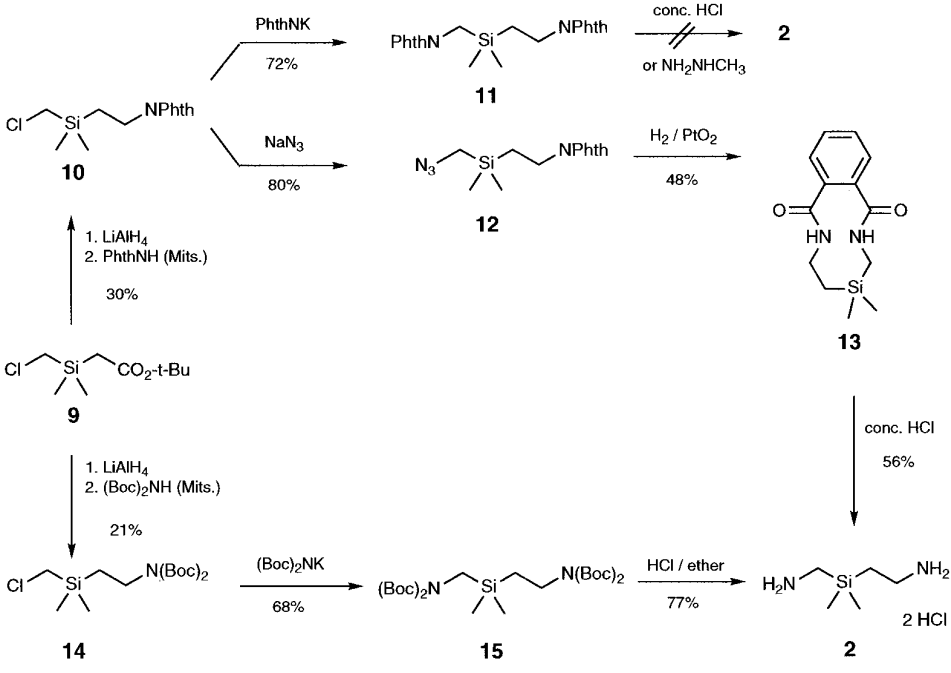
The synthesis of the symmetrical diamines **1** and **5** was performed as described in Scheme 4. The volatile diamine obtained by catalytic hydrogenation of diazide **7a** was difficult to purify (see also *18*); to avoid this problem, the di-BOC derivative was formed *in situ* by direct reaction of di-BOC-dicarbonate with the hydrogenation mixture, subsequent acidic deprotection giving compound **1** as a crystalline salt, in reasonable yield. A similar approach was used to prepare diamine **5** (in this case, **8b** was obtained in two steps from **7b**).

Several approaches to the desired silaputrescine **2** were tried, as described in Scheme 5. The key intermediate, ester **9,** was prepared in 83% yield from chloro-(chloromethyl)dimethylsilane and *tert*-butyl acetate following a published procedure (*19*). In the first approach, the diphthalimido derivative **11** could be obtained in three steps: reduction of ester **9** to the corresponding alcohol derivative with lithium aluminum hydride, transformation of the alcohol to monophathlimide derivative **10** by the Mitsunobu procedure, and finally introduction of the second phthalimido group using the Gabriel approach. The highly insoluble **11** could not be efficiently deprotected to the desired diamine **2,** neither by refluxing in concentrated HCl nor by treatment with methylhydrazine, where only traces of **2** could be detected

6a	80%	**7a**	27% (1 step)	**8a**	58%	**1**
6b	90%	**7b**	25% (2 steps)	**8b**	67%	**5**

a : X = $Si(CH_3)_2$
b : X = $Si(CH_3)_2$-O-$Si(CH_3)_2$

SCHEME 4

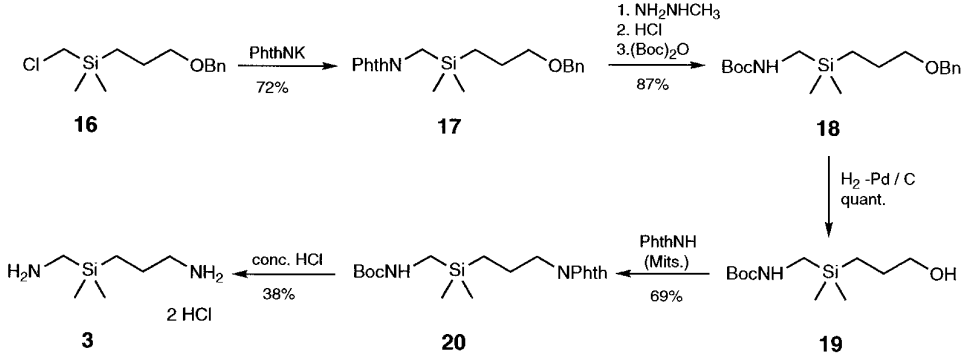


SCHEME 5

(instability of **2** under basic conditions). Alternatively, to overcome the solubility problem of **11,** the monophthalimido-containing azide derivative **12** was prepared; hydrogenation of the latter did not give the desired 1-[2-(*N*-phthalimido)ethyl]-aminomethyldimethylsilane but resulted in the undesired cyclic derivative **13** (spectroscopic data in accordance with its structure; see Experimental Procedures). Silaputrescine **2** was successfully obtained by refluxing compound **13** in concentrated HCl. Finally, a more straightforward approach gave diamine **2** in better overall yield: the tetra-BOC-diamine **15** was prepared using chemical procedures similar to those used to obtain diphthalimido derivative **11,** di-*tert*-butyliminodicarboxylate replacing phthalimide in the Mitsunobu- and Gabriel (potassium salt)-type reactions. Deprotection of **15** under mild acidic conditions afforded the desired silaputrescine, dihydrochloride **2** in 77% yield.

The asymmetric silacadaverine **3** was synthesized according to Scheme 6. The propyl side chain was attached to the silicon atom by condensation of the Grignard reagent derived from 3-benzyloxy-1-chloropropane and chloro(chloromethyl)dimethylsilane. Stepwise introduction of a BOC-protected amine moiety afforded compound **18.** Deprotection to alcohol **19** and Mitsunobu reaction with phthalimide gave the bis-protected diamine **20,** subsequently transformed to the desired diamine dihydrochloride **3** (refluxing in concentrated HCl).

Synthesis of the symmetrical silacadaverine **4** is described in Scheme 7. The unsaturated bis-BOC-protected amine **23** was prepared according to the procedure used for the obtention of compound **14** (Scheme 5). Epoxidation of vinylsilane **23**

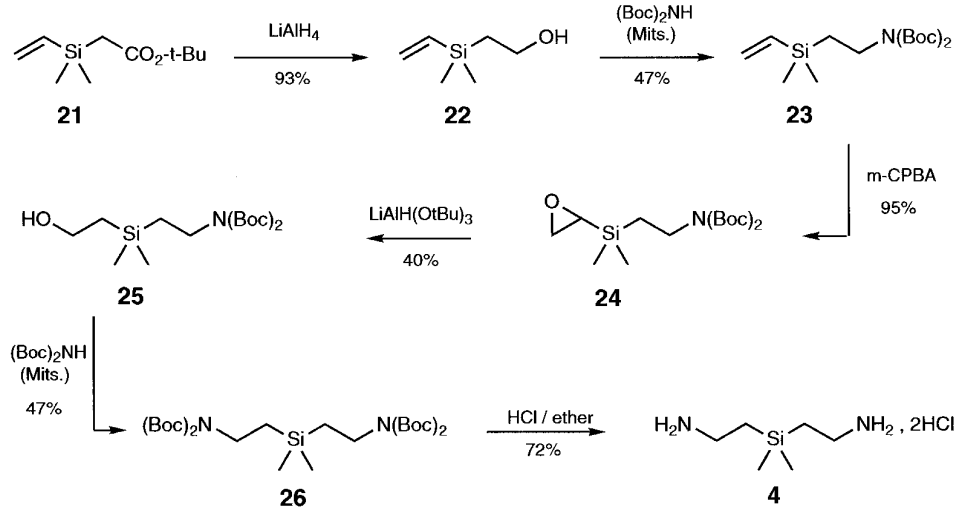


SCHEME 6

gave epoxide **24** in very good yield (95%). Selective conversion of α-epoxysilanes to β-hydroxysilanes is known to occur with lithium aluminum hydride (*20*), but only traces of **25** could be detected with this reagent due to the reactivity of the bis-BOC-protected amine (loss of one BOC group in one identified byproduct). Alcohol **25** was obtained in an acceptable yield with the attenuated reducing agent, tri(*tert*-butoxy)lithium aluminum hydride. Silacadaverine **4** was obtained as its dihydrochloride salt in 72% yield by deprotection of the tetra-BOC diamine **26** in HCl/diethyl ether.

Biochemistry

Compounds **1–5** were tested as potential time-dependent inhibitors of diamine oxidases prepared from hog kidney and rat small intestine. At concentrations as



SCHEME 7

high as 10 mM, **2, 4,** and **5** did not produce any enzyme inactivation. In addition, it was checked that **2, 4,** and **5** were neither reversible inhibitors nor substrates of the enzymes when tested at 1 mM. On the contrary, incubation of either enzyme with **1** or **3** resulted in time-dependent loss of diamine oxidase activity which followed first-order kinetics. Loss of activity was related to inhibitor concentration. By plotting the time of half-inactivation ($t_{1/2}$) as a function of the reciprocal of inhibitor concentration ($1/I$) according to the method of Kitz and Wilson (*21*), a straight line was obtained. Except in the case of the inactivation of rat small intestine diamine oxidase by **3,** the line did not pass through the origin but intercepted the positive *y* axis, demonstrating a saturation effect which involves the enzyme active site in the inhibitory process. Kinetic constants for the time-dependent inhibition of the enzymes, i.e., the apparent dissociation constant (K_I) and the times of half-inactivation to infinite concentration of inhibitor ($\tau_{1/2}$), could be extrapolated from such Kitz and Wilson replots (see Table 1).

Further studies on diamine oxidases by **1** and **3** showed protective effects of putrescine, a substrate of the enzymes. This confirms that the inactivation takes place in the active site. Furthermore, addition of 2-mercaptoethanol (5 mM) to the preincubation medium did not modify inactivation rates. These results, as well as the absence of a lag time before the onset of inhibition, rule out the possibility that the species responsible for inactivation were released from the enzyme active site (*22*). Incubation of hog kidney diamine oxidase with 1 mM **1** for 40 min at 37°C resulted in 75% inactivation of the enzyme. Prolonged (24 h) dialysis at 4°C of the inactivated enzyme produced a partial regeneration (20%) of enzyme activity, suggesting that covalent linkage of the inhibitor to the enzyme, if any, is slowly reversible. Qualitatively similar results were observed for compound **3.**

Discussion

Our results demonstrate that the silane diamines **1** and **3** are mechanism-based inhibitors of diamine oxidases from hog kidney and rat small intestine. However, it is noteworthy that compounds **2, 4,** and **5** do not produce time-dependent inhibition. Compound **4** was expected to produce inactivation of diamine oxidase through pathways shown in Scheme 3; furthermore, there are precedents for amine oxidase

TABLE 1
Kinetic Constants of Diamine Oxidase Irreversible Inhibitors

	Hog kidney diamine oxidase		Rat small intestine diamine oxidase	
Compound	K_I (m*M*)	$\tau_{1/2}$ (min)	K_I (m*M*)	$\tau_{1/2}$ (min)
1	3.0 ± 0.6	0.6 ± 0.1	1.8 ± 0.5	0.5 ± 0.1
3	13 ± 6	1.8 ± 1.1	No saturation kinetics ($t_{1/2}$ = 6.7 min at 5 m*M*)	

inhibition by (amino*ethyl*)trimethylsilanes or analogs (*1*, *9*). Two possible reasons may explain the lack of activity of **4:** either the dimethylsilane group in the β-position of the amine produces a steric hindrance which impairs any binding to the enzyme or the pathways in Scheme 3 are not compatible with the enzyme mechanism. The fact that **4** is neither a reversible inhibitor nor a substrate for the two enzymes (at least up to 1 mM) would favor the first alternative. If the steric hindrance of the dimethylsilane moiety in the β-position of the amine is the cause of the lack of activity of **4,** then compound **2,** which is asymmetrical, may be oriented as **4** in the active site. The absence of activity of **5** could also be explained by steric hindrance (two dimethylsilyl groups in the structure).

That the inactivation of diamine oxidases by **1** appears to be slowly reversible upon prolonged dialysis would favor path b, and even b_1 (Scheme 2) by analogy with the mechanisms demonstrated by Banik and Silverman (*3*) for the inactivation of bovine MAO-B by (amino*methyl*)trimethylsilane. The inactivation of diamine oxidases by **3** could be similar to that produced by **1** if the compound interacts as a Schiff base with the cofactor on the amino*methyl* side. If the Schiff base is formed between the cofactor and the less basic (*23*) amino*propyl* side of **3,** a radical mechanism might be involved, in analogy with the mechanism proposed for the inactivation of bovine MAO-B by (amino*propyl*)trimethylsilane. A radical mechanism has been recently suggested for the plasma amine oxidase catalysis (*24*). Additional work will be required to further clarify the nature of interaction of silane diamines with diamine oxidases.

In conclusion, our results show that it is possible to design silane diamines which produce inactivation of diamine oxidases from two different sources. In this respect, our results extend and generalize the concept of silicon-based inactivation of amine oxidases.

EXPERIMENTAL PROCEDURES

Material and General

Anhydrous solvents were obtained as follows: diethyl ether and tetrahydrofuran were distilled from sodium/benzophenone; dimethylformamide and diisopropylamine were distilled from calcium hydride. All the following commercially available reagents were used as obtained. 1,3-Bis(chloromethyl)tetramethyldisiloxane and vinyldimethylchlorosilane were purchased from Petrarch Systems; bis(chloromethyl)dimethylsilane, chloro(chloromethyl)dimethylsilane, sodium azide, di-*tert*-butyldicarbonate, and diethylazodicarboxylate from Aldrich; lithium aluminum hydride, platinum oxide, and diisopropylazodicarboxylate from Janssen Chimica; di-*tert*-butyliminodicarboxylate and 10% palladium on activated charcoal from Fluka; *tert*-butyl acetate, butyllithium, phthalimide, potassium phthalimide, lithium aluminum tri(*tert*-butoxy)hydride, and triphenylphosphine from Merck–Schuchardt.

Thin-layer chromatography and flash chromatography refer to silica gel chromatography (Merck 60 PF254 plates and kieselgel 60, 230–300 mesh). The BOC-protected amines and amine derivatives were visualized with ninhydrin, the other