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Novel 3,4-Diazabenzotropone Compounds (2,3-Benzodiazepin-5-ones): Synthesis, Unique Reactivity, and Biological Evaluation

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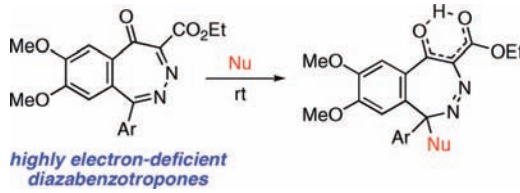
ABSTRACT



Efficient synthesis of 3,4-diazabenzo[*b*]tropone was first achieved utilizing 4π−8π sequential electrocyclic reactions of functionalized benzocyclobutenone derivatives. These compounds are highly electron deficient and readily form amine adducts at ambient temperature. Furthermore, gentle heating resulted in quantitative nitrogen extrusion to produce indenone derivatives. These diazabenzotropones were found to exhibit potent apoptosis-inducing activity against human lymphoma cells. Thus, novel amine-catalyzed nitrogen extrusion reactions and interesting bioactivities were found to be characteristic of these novel diazabenzotropone compounds.

The 2,3-benzodiazepine nucleus constitutes the partial structure of an important class of bioactive molecules known as noncompetitive 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)propionic acid (AMPA) receptor antagonists, which exert anticonvulsant and neuroprotective activities.[1] To date, numerous derivatives have been synthesized and biologically evaluated, and most of these utilize 2,3-benzodiazepin-4-one derivatives as a key synthetic intermediate.[2] On the other hand, we recently reported a novel strategy for the synthesis of 2,3-benzodiazepin-5-one derivatives based on the oxyanion-accelerated successive electrocyclic reactions of benzocyclobutenones.[3] Surprisingly, an online search for 2,3-benzodiazepine skeletons having a 5-oxo group resulted in no hits, indicating that ours is the first example for the synthesis of such benzodiazepine derivatives, probably because an approach involving the formal insertion of diazomethylene into the C−C bond of benzocyclobutenone is quite unique. We then focused on the synthesis and characterization of corresponding diazabenzotropone derivatives that have not been reported to date.[4] Because the tropone ring system and its aza analogue have been discussed

(1) For reviews, see: (a) Zappala, M.; Grasso, S.; Micale, N.; Polimeni, S.; De Micheli, C. *Mini-Rev. Med. Chem.* **2001**, *1*, 243–253. (b) Solyom, S.; Tarnawa, I. *Curr. Pharm. Design* **2002**, 913–939.

(2) For recent examples, see: (a) Zappala, M.; Postorino, G.; Micale, N.; Caccamese, S.; Parrinello, N.; Grazioso, G.; Roda, G.; Menniti, F. S.; De Sarro, G.; Grasso, S. *J. Med. Chem.* **2006**, *49*, 575–581. (b) Elger, B.; Huth, A.; Neuhaus, R.; Ottow, E.; Schneider, H.; Seilheimer, B.; Turski, L. *J. Med. Chem.* **2005**, *48*, 4618–4627. (c) Gitto, R.; Orlando, V.; Quartarone, S.; De Sarro, G.; De Sarro, A.; Russo, E.; Ferreri, G.; Chimirri, A. *J. Med. Chem.* **2003**, *46*, 3758–3761. (d) Szabados, T.; Gigler, G.; Gacsalyi, I.; Gyertan, I.; Levay, G. *Brain Res. Bull.* **2001**, *55*, 387–391.

(3) Matsuya, Y.; Ohsawa, N.; Nemoto, H. *J. Am. Chem. Soc.* **2006**, *128*, 13072–13073.

(4) For a recent report on the synthesis of monocyclic 2-azatropone, see: (a) Takami, S.; Oshida, A.; Tawada, Y.; Kashino, S.; Satake, K.; Kimura, M. *J. Org. Chem.* **2000**, *65*, 6093–6096. Recently, a diazabicyclic benzotropolone framework has been reported; see: (b) Khrizman, A.; Moulthrop, J. S.; Little, S.; Wharton, H.; Yardley, V.; Moyna, G. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4183–4186.

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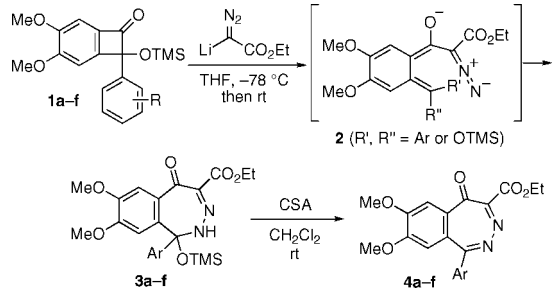
Scheme 1



in regard to aromaticity due to polarization of the carbonyl group,[5] the chemical reactivity and biological action of this new aza-tropone system is of great interest. Thus, we achieved the first synthesis of a series of 3,4-diazabenzo-[*b*]tropone compounds, 1-aryl-2,3-benzodiazepin-5-one-4-carboxylates (**4**), employing 1-silyloxybenzocyclobuten-1-ones (**1**) as a substrate in the sequential electrocyclic reactions. While diazabenzotropone series **4** was thermally stable, these compounds were found to be highly susceptible to nucleophilic species and easily formed the corresponding addition products. Herein, we report a novel catalytic nitrogen extrusion reaction of 2,3-benzodiazepin-5-one-4-carboxylate triggered by amine-adduct formation and preliminary evaluation of bioactivity lying in these novel diazabenzotropones.

Table 1. Transformation of Benzocyclobutenones (**1**) into 2,3-Benzodiazepines (**4**)

entry	substrate	R	yield (%) of **3**	yield (%) of **4**
1	**1a**	H	75	87
2	**1b**	4-Me	69	94
3	**1c**	2-Me	81	84
4	**1d**	4-MeO	64	99
5	**1e**	4-Br	71	88
6	**1f**	4-CF_3	44[a]	95

[a] 50% of the starting benzocyclobutenone (**1f**) was recovered.

Syntheses of the 2,3-benzodiazepin-5-one derivatives (**4a**−**f**) are summarized in Scheme 1 and Table 1. Substituted benzocyclobutenone **1a**−**f**[6] were allowed to react with lithiated diazoacetate at −78 °C and were then warmed to room temperature. As discussed in a previous report,[3] the oxide anion generated by diazoacetate addition facilitated 4π electrocyclic ring opening of the cyclobutene and exhibited strong outward torquoselectivity to form *o*-quinodimethane intermediate **2**. Subsequent 1,7-electrocyclization and protonation gave diazepines **3a**−**f** in satisfactory yields.

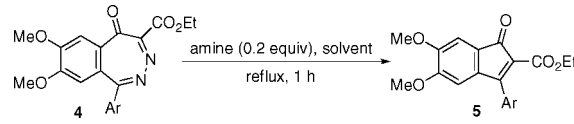
(5) For recent topics on benzotropones, see: (a) Ohkita, M.; Sano, K.; Suzuki, T.; Tsuji, T.; Sato, T.; Niino, H. *Org. Biomol. Chem.* **2004**, *2*, 1044–1050. (b) Güney, M.; Dastan, A.; Balci, M. *Helv. Chim. Acta* **2005**, *88*, 830–838. (c) Ohkita, M.; Sano, K.; Suzuki, T.; Tsuji, T. *Tetrahedron Lett.* **2001**, *42*, 7295–7297.

(6) Preparation of these compounds is described in the Supporting Information.

Treatment of these compounds with camphorsulfonic acid caused a silanol elimination to afford the diazatropone-type compounds **4a**−**f** in good yields.

During the examination of several chemical transformations of diazepine series **4**, we noticed that these compounds were prone to decompose with loss of nitrogen under certain conditions, particularly with heating. Careful investigations revealed that the degradation was caused by amino compounds, leading to the formation of indenone derivatives (**5**) (Scheme 2).

Scheme 2



Although the diazepine **4a** was stable in refluxing benzene, as shown in Table 2 (entry 1), the situation changed markedly in the presence of small amount of benzylamine. In various solvents, nitrogen extrusion occurred to produce the indenone derivative **5a** in different yields (entries 2−6). Considerable amounts of benzylamide (arising from ester−amide transformation) were detected in entries 2−4, making the reaction system somewhat complex due to consumption of the reagent (benzylamine). In DMF or EtOH, the reactions were free from such problems, and clean conversion to **5a** was observed without any side reactions. Instead of benzylamine, another aliphatic primary amine such as methylamine also

Table 2. Nitrogen Extrusion Reaction of 2,3-Benzodiazepin-5-ones (**4a**−**f**)

entry	substrate	solvent	amine	yield (%)[a]
1	**a**	benzene	none	0
2	**a**	benzene	$BnNH_2$	24
3	**a**	DME[b]	$BnNH_2$	30
4	**a**	DCE[c]	$BnNH_2$	66
5[d]	**a**	DMF	$BnNH_2$	88
6	**a**	EtOH	$BnNH_2$	93
7	**a**	EtOH	$MeNH_2$	89
8	**a**	EtOH	$PhNH_2$	26[e]
9	**a**	EtOH	pyrrolidine	59[e]
10	**a**	EtOH	Et_3N	0[e]
11	**a**	EtOH	DMAP	0[e]
12	**a**	EtOH	*t*-BuOK	0
13	**a**	EtOH	Bu_3P	0
14	**b**	EtOH	$BnNH_2$	97
15[f]	**c**	EtOH	$BnNH_2$	47
16	**d**	EtOH	$BnNH_2$	quant.
17	**e**	EtOH	$BnNH_2$	quant.
18	**f**	EtOH	$BnNH_2$	quant.

[a] Entries 14−18 represent isolation yields. The others were estimated by NMR spectra. [b] 1,2-Dimethoxyethane. [c] 1,2-Dichloroethane. [d] The reaction was carried out at 90 °C. [e] The starting diazepine was recovered. [f] The reaction was continued for 8 h.

gave a good yield (entry 7). The reaction rate was considerably lower when using aniline (entry 8) or secondary amine (entry 9), probably due to a lower nucleophilicity and a steric hindrance, and the reaction did not proceed at all in the presence of tertiary amines (entries 10 and 11). The other nucleophiles (*t*-BuOK or Bu_3P) did not afford **5a**, leading to the formation of rather complicated mixture (entries 12 and 13). Under optimal conditions, the effects of substituents on the 1-aryl group were examined (entries 14−18). It was confirmed that nitrogen extrusion occurred in nearly quantitative yields, regardless of electron-donating or -withdrawing substituents. The only exception was the case of the 2-tolyl substrate **4c** (entry 15), which gave the indenone **5c** in 47% yield after a prolonged reaction time. The discontinuity of the reactivity observed here is considered to originate in the steric hindrance of the ortho substituent, suggesting that the key interaction between diazepine and amine leading to nitrogen extrusion takes place at or around the 1-position.

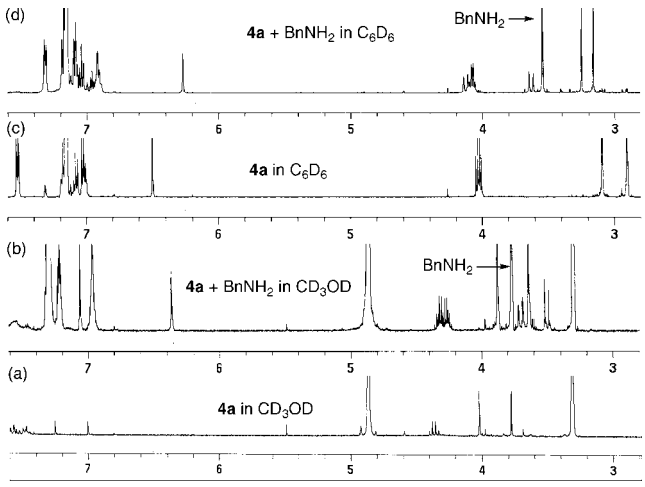
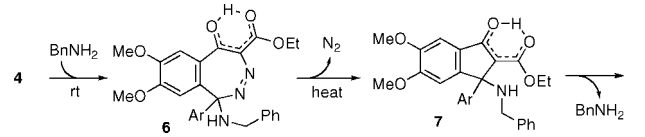


Figure 1. ^{1}H NMR spectra of **4a** before and after addition of benzylamine.

The seven-membered ring of the 2,3-benzodiazepin-5-one derivatives **4** may be highly electron-deficient because of the presence of two carbonyl groups and two electronegative nitrogen atoms. This is likely to make these compounds highly reactive toward nucleophilic reagents. Actually, ^{1}H NMR analyses of **4a** (+ $BnNH_2$) demonstrated quantitative formation of an addition compound (Figure 1). Addition of a slight excess of $BnNH_2$ to a solution of **4a** in CD_3OD resulted in the complete disappearance of **4a**, new benzyl methylene protons with a geminal coupling (J = 15 Hz) were seen (3.5−3.7 ppm), and the methylene protons of ethyl ester lost equivalence (Figure 1a,b). We presumed that these spectral changes were caused by the formation of addition compound **6** (Scheme 3).[7] This transformation was completed instantaneously at room temperature, and no changes were observed after a further 1 h.

(7) The adduct **6** is likely to be stable only in a solution. Attempts to isolate **6** ended in failure because regeneration of the diazepine **4** took place during the isolation process.

Scheme 3



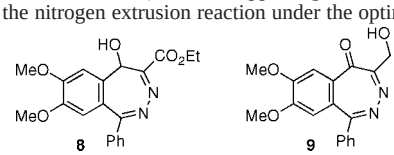
However, gentle heating of this solution led to conversion to indenone **5**, suggesting the thermal instability of adduct **6**, probably due to ready release of the stable nitrogen molecule.[8] In this connection, the same adduct formation was likely to occur in a less polar C_6D_6 solvent at room temperature (Figure 1c,d), although thermolysis gave a somewhat complex mixture in this case (vide supra). After extrusion of nitrogen, $BnNH_2$ was regenerated to release the indenone product **5** and achieve a catalytic cycle (Scheme 3). Although examples of nitrogen extrusion from a 2,3-benzodiazepine nucleus have been found in the literature, these reactions have only been described as minor reaction modes of several degradation and isomerization paths under thermolytic or photolytic conditions.[9] It is important to note that indenone **5** did not produce its adduct form **7** in a reversible manner. Thus, the amine-adduct formation at ambient temperature is a novel characteristic of diazabenzotropone derivatives **4**.[10]

With the observation of unique reactivity of diazabenzotropones **4** toward nucleophiles, our interest turned to their biological behaviors. Their highly electron-deficient character was associated with the ability of a strong interaction with electron-donating substances in living cells. In fact, we could observe an instantaneous reaction of **4a** with *N*-Boc-cysteine methyl ester as an example of a biomolecule component, in a ^{1}H NMR analysis. Although this did not guarantee a bioactivity of diazabenzotropones, we considered that examinations of biological activities of **4** may be meaningful. We have recently reported intracellular oxidative stress-triggered apoptosis of human lymphoma cells, which is more sharply induced by small molecule natural products (macrosphelides) having a higher oxidation state (i.e., higher electron deficiency) rather than the relatively lower oxidation state.[11] Aside from the structural irrelevancy to these natural compounds, the diazabenzotropones

(8) Recently, decomposition of 1,2,3-triazolines with loss of nitrogen to form aziridines has been reported; see: Kim, S.; Lee, Y. M.; Lee, J.; Lee, T.; Fu, Y.; Song, Y.; Cho, J.; Kim, D. *J. Org. Chem.* **2007**, *72*, 4886–4891.

(9) (a) Reid, A. A.; Sood, H. R.; Sharp, J. T. *J. Chem. Soc., Perkin Trans. 1* **1976**, 362–366. (b) Munro, D. P.; Sharp, J. T. *J. Chem. Soc., Perkin Trans. 1* **1980**, 1718–1723. (c) Reid, A. A.; Sharp, J. T.; Murray, S. J. *J. Chem. Soc., Chem. Commun.* **1972**, 827.

(10) Less electron deficient derivatives **8** and **9**, which were prepared by $NaBH_4$ reduction of **4a** (see the Supporting Information), did not participate in the nitrogen extrusion reaction under the optimal conditions.



(11) (a) Ahmed, K.; Zhao, Q.-L.; Matsuya, Y.; Yu, D.-Y.; Salunga, T. L.; Nemoto, H.; Kondo, T. *Int. J. Hyperthermia* **2007**, *23*, 353–361. (b) Ahmed, K.; Zhao, Q.-L.; Matsuya, Y.; Yu, D.-Y.; Feril, L. B., Jr; Nemoto, H.; Kondo, T. *Chem. Biol. Interact.* **2007**, *170*, 86–99. (c) Matsuya, Y.; Kawaguchi, T.; Ishihara, K.; Ahmed, K.; Zhao, Q.-L.; Kondo, T.; Nemoto, H. *Org. Lett.* **2006**, *8*, 4609–4612.

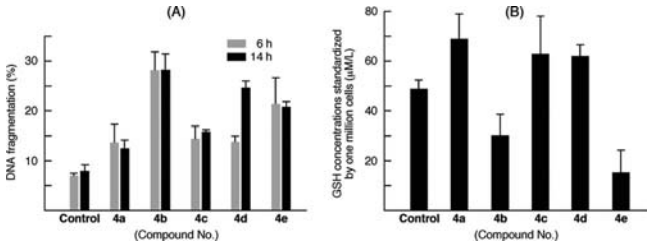


Figure 2. Effects of diazabenzotropone compounds **4** on DNA fragmentation (A) and intracellular GSH concentrations (B) of human lymphoma U937 cells at 10 μM drug concentration. Incubation time is 6 and 14 h for (A) and 6 h for (B). The results are presented as the mean $\pm$ SD ($n = 3$).

4 were expected to show an apoptosis-inducing activity as a result of an interaction with intracellular oxidative stress-controlling substances such as glutathione (GSH), which acts as an intracellular antioxidant. Thus, the diazabenzotropones **4** were studied to evaluate their DNA fragmentation ability (one of the significant characteristics of apoptosis) against U937 human lymphoma cells, and the results are summarized in Figure 2A. The percentage of the DNA fragmentation cell was determined by the previously reported method.[11] Gratifyingly, the clear activity was observed for some of the tested compounds after 6 or 14 h incubation at 10 μM concentration, and the compound **4b** showed maximum potency. In additional preliminary examinations, it has been revealed that these compounds are able to increase an intracellular concentration of the reactive oxygen species (data not shown). Encouraged by these findings, we further investigated effects of these compounds on the intracellular GSH concentration using a standard assay protocol.[12] After 6 h incubation at 10 μM concentration, the compounds **4b** and **4e** obviously brought about significant decrease of the intracellular GSH level (Figure 2B), which was parallel to the DNA fragmentation data for this series of compounds. These experimental results strongly suggest that diazabenzotropones synthesized in this study can potentially exert an apoptosis-inducing activity against tumor cell lines, probably due to the intracellular oxidative stress induction caused by lowering of the GSH level. Similarity of the biological profiles toward apoptotic cell death between diazabenzotropones and macrosphelides still remains unclear. Although further studies are required to elucidate the intracellular action mechanisms of diazabenzotropones, we believe that the extremely high reactivity of the diazabenzotropone nucleus as an electron acceptor plays an important role in their bioactivities.

In this paper, we describe the first synthesis of novel diazabenzotropone compounds and their unique chemical and biological characters. The remarkably facile amine-adduct formation and the subsequent nitrogen extrusion reaction are the main features of this series of compounds. The high electron deficiency of the diazatropone ring is likely to make such a novel amine-catalyzed process possible. It is noteworthy that the adduct formation can occur in a quantitative manner at ambient temperature. Interesting apoptosis-inducing activity of diazabenzotropones may be attributed to such a high electron-accepting ability and possible interactions with nucleophilic biomolecules such as GSH. Further chemical applications and biological studies of the novel diazabenzotropone compounds synthesized in this study will be performed and reported in the near future.

Supporting Information Available: Experimental procedures, compound characterization data, and ^{1}H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(12) (a) Griffith, O. W. *Anal. Biochem.* **1980**, *106*, 207–212. (b) Baker, M. A.; Cerniglia, G. J.; Zaman, A. *Anal. Biochem.* **1990**, *190*, 360–365. (c) Vandeputte, C.; Guizon, I.; Genestie-Denis, I.; Vannier, B.; Lorenzon, G. *Cell Biol. Toxicol.* **1994**, *10*, 415–421.