Regioselective Lithiation and Functionalization of 3-(Benzyloxy)isothiazole: Application to the Synthesis of Thioibotenic Acid

Lennart Bunch, Povl Krogsgaard-Larsen, and Ulf Madsen*

Department of Medicinal Chemistry, The Royal Danish School of Pharmacy, Universitetsparken 2, DK-2100 Copenhagen, Denmark

um@dfh.dk

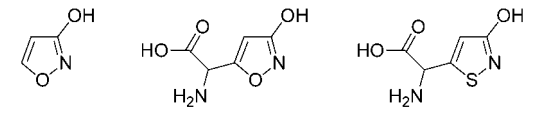
Received October 18, 2001

Abstract: Direct functionalization of the 3-oxygenated isothiazole heteroaromatic parental system has not yet been reported in the literature. Here, we report the first regioselective lithiation of the 5-position of 3-(benzyloxy)isothiazole (**4**) using LDA in diethyl ether. The versatility of the methodology was explored by quenching with a variety of electrophiles to give the desired products **7a**,**b**,**d**−**g** in 54−68% yield. Only benzoylation aiming at the synthesis of **7c** was unsuccessful. Furthermore, a highly convergent synthesis of thioibotenic acid (**1**), the sulfur analogue of the neurotoxic natural product ibotenic acid, was carried out.

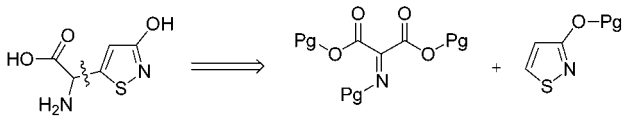
Bioisosteric modification of biological active molecules is a well-described and often used strategy in medicinal chemistry research. The 3-hydroxyisoxazole[1] moiety is an extensively used carboxylic acid bioisostere.[2,3] It is also found in the naturally occurring amino acid ibotenic acid,[4,5] a widely used neurotoxin and pharmacological tool for studies of glutamic acid receptors (Scheme 1). Substitution of sulfur for the ring oxygen provides the parental system 3-hydroxyisothiazole, which is less acidic (pK_a ∼7) and more lipophilic compared with the 3-hydroxyisoxazole group (pK_a ∼5).[6] On this basis, we were interested in synthesizing thioibotenic acid (**1**), the sulfur analogue of ibotenic acid (Scheme 1).

A retro-synthetic analysis of thioibotenic acid (**1**) suggests the formation of the sp^2−sp^3 carbon−carbon bond as the key step (Scheme 2). A possible strategy would thus be selective generation of the 5-anion of suitably protected **3**, followed by the addition to an imine **2**.[7] However, in contrast to the extensive research in the field of direct functionalization of heteroaromatic rings, no methodology has so far been reported for the functionalization of the 3-oxygenated isothiazole parental system.

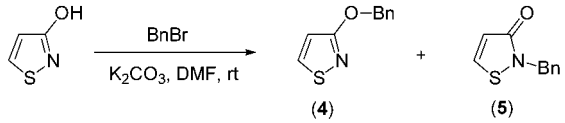
Scheme 1



Scheme 2



Scheme 3



The synthesis of 3-hydroxyisothiazole can be achieved in two steps from commercially available 3,3′-dithianedipropionic acid in moderate yield.[8] Benzylation provides an easily separable mixture of the O- and N-benzylated derivatives **4** and **5**, respectively (90% yield, ratio 2:1), which is in agreement with a previous study[9] (Scheme 3). Selective lithiation of the 5-position of isothiazole has been accomplished using *n*-BuLi in diethyl ether.[10] However, this strategy failed completely in the case of **4**, presumably due to competing nucleophilic addition of *n*-BuLi to the hetereoaromatic ring. It was therefore decided to explore the use of more sterically hindered lithium amide bases. Initially, the influence of solvent on stability of the 5-lithio anion of **4** generated from LDA at −78 °C was investigated. In diethyl ether, immediate precipitation and high stability of this salt was evident, while in THF no precipitation was observed, and instead, decomposition proved to be progressing over time. Whereas only one pathway for decomposition of 5-lithio isothiazole seems reasonable, two plausible pathways can be proposed for the decomposition of 5-lithio 3-(benzyloxy)-isothiazole (Scheme 4). Only benzyl alcohol was identified as a byproduct when the reaction was run in diethyl ether or in THF. No trace of benzyloxynitrile or the acid-catalyzed hydrolysis product carbamic acid benzyl ester[11,12] was detected in the crude product mixture. Thus, we suggest that path b is the preferred pathway of decomposition.

We then turned to investigate the influence of the steric bulk and strength of the lithium amide base and reaction time on the percentage of deuterium incorporation. However, only partial incorporation could be achieved using lithium ethylisopropyl amide (LEIA) or LDA, which

(1) Sørensen, U. S.; Krogsgaard-Larsen, P. *Org. Prep. Proced. Int.* **2001**, *33*, 515−564.
(2) Krogsgaard-Larsen P.; Frølund, B.; Kristiansen, U.; Frydenvang, K.; Ebert, B. *Eur. J. Pharm. Sci.* **1997**, *5*, 355−384.
(3) Madsen, U.; Sløk, F. A.; Johansen, T. N.; Ebert, B.; Krogsgaard-Larsen, P. *Curr. Top. Med. Chem.* **1997**, *2*, 1−14.
(4) Eugster, C. H. In *Fortschritte der Chemie Organischer Naturstoffe XXVII*; Zechmeister, L., Ed.; Springer-Verlag, New York, 1969; p 261.
(5) Krogsgaard-Larsen, P.; Honoré, T.; Hansen, J. J.; Curtis, D. R.; Lodge, D. *Nature* **1980**, *284*, 64−66.
(6) Frydenvang, K.; Matzen, L.; Norrby, P.-O.; Sløk, F. A.; Liljefors, T.; Krogsgaard-Larsen, P.; Jaroszewski, W. *J. Chem. Soc., Perkin Trans. 2* **1997**, 1783−1791.
(7) Recently, the synthesis of several α-aryl and α-heteroaryl glycine derivatives has been reported based on the addition of lithiated aryl and heteroaryl moieties to imine **6**. Calí, P.; Begtrup, M. *Synthesis* **2002**, 63−66.
(8) Beeley, N. R. A.; Harwood: L. M.; Hedger, P. C. *J. Chem. Soc., Perkin Trans. 1* **1994**, 2245−2251 and references therein.
(9) Chan, A. W. K.; Crow, W. D.; Gosney, I. *Tetrahedron* **1970**, *26*, 2497−2506.
(10) Iddon, B. *Heterocycles* **1995**, *41*, 533−593.
(11) Grigat, E.; Puetter, R. *Chem. Ber.* **1964**, *97*, 3018−3021.
(12) Buckley, N.; Oppenheimer, N. J. *J. Org. Chem.* **1997**, *62*, 540−551.

10.1021/jo0162134 CCC: $22.00
Published on Web 03/03/2002

Scheme 4

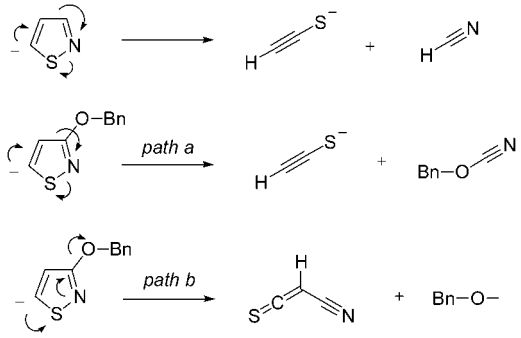
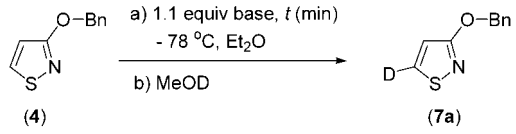


Table 1. Investigation of Effects of Lithium Amide Base and Time on the Percentage of Deuterium Incorporation[a] and Degree of Decomposition[b]



(**4**) (**7a**)

	t = 15 min			t = 45 min		
base	**4** (%)	**7a** (%)	BnOH	**4** (%)	**7a** (%)	BnOH
LDA	38	57	5	38	57	5
LEIA	67	28	<5	40	60[c]	0
LTMP	86	9	5	32	14	55

[a] The percentage of deuterium incorporation was determined from ^{1}H NMR. [b] The percentage of decomposition was determined from the amount of BnOH formed. [c] Extending the reaction time to 2 h, a further increase in deuterium incorporation was *not* observed.

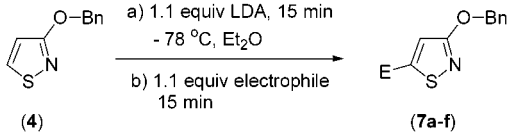
could be due to the setup of an equilibrium between the dialkylamine and the 5-lithiated heterocycle. Surprisingly, the use of the more basic and more sterically hindered amide base, lithium 2,2,6,6-tetramethylpiperidine (LTMP), resulted in extensive decomposition (Table 1). Further studies with LDA as the base were therefore carried out. Performing the reaction with inverse or forward addition of the LDA base did not influence the reaction outcome significantly. When the reaction was run at half the concentration (0.05 M), a slight increase in yield (67%) was observed, whereas the use of 2.2 equiv and 4.0 equiv of LDA gave 76% and 86% incorporation of deuterium, respectively. However, a large excess of LDA may pose a problem on the following reaction with some electrophiles. Also, addition of a cosolvent, *N*,*N*-dimethylpropyleneurea (DMPU, 30 Vol %), was tried but resulted only in extensive decomposition.

To investigate the generality of this new methodology for introduction of substituents in the 5-position of 3-(benzyloxy)isothiazole (**4**), a series of electrophiles were chosen. In the presence of 1.1 equiv of LDA in a 0.1 M diethyl ether solution (Table 2), the desired products **7b**,**d**−**f** could be isolated in 54−68% yields.[13] However, attempted benzoylation using benzoyl chloride or benzoyl cyanide to give **7c** proved to be unsuccessful and resulted in a complex reaction mixture.

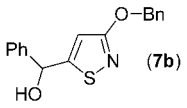
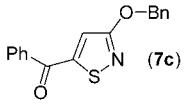
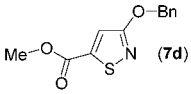
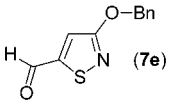
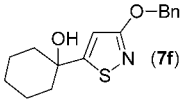
With this new methodology for functionalization of the 3-oxygenated isothiazole parental system in hand, our

(13) All yields given are isolated yields after chromatography. In all cases, a maximum of 5% decomposition was observed. To improve the efficiency of the reaction, recovery of starting material **4** could be achieved. Also, lowering the concentration of the reaction components and use of larger excess of LDA may be advantageous, as observed in the deuterium experiments.

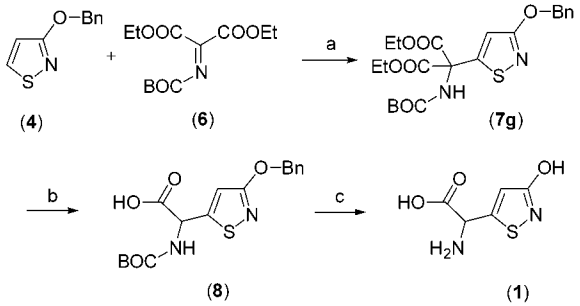
Table 2. Reaction of 4 with Various Electrophiles Using the General Procedure



(**4**) (**7a-f**)

Electrophile	Product	Yield [13]
PhCHO	 (**7b**)	65 %
PhCOCl or PhCOCN	 (**7c**)	0 %
MeOCOCN	 (**7d**)	56 %
DMF	 (**7e**)	54 %
Cyclo-hexanone	 (**7f**)	68 %

Scheme 5[a]



(**4**) (**6**) (**7g**) (**8**) (**1**)

[a] Reagents and reaction conditions: (a) 1.1 equiv of LDA, −78 °C, Et_2O, then 1.1 equiv of imine **6** (56%); (b) LiOH, H_2O/THF, rt, 4 h (76%); (c) 10% HBr/AcOH, rt, 2 h (60%).

next step was to apply it to the synthesis of thioibotenic acid (**1**) (Scheme 5). The 5-lithio anion of **4** was generated using 1.1 equiv of LDA and subsequently reacted with imine **6** to give the desired product **7g** in 56% yield.[14] The following hydrolysis of the ester functionalities also mediated mono-decarboxylation upon the acidic workup to give crude **8** in 76% yield. Cleavage of the benzyl ether and simultaneous removal of the BOC group were accomplished in HBr/AcOH to give **1** as the zwitterion in 60% yield after treatment with Et_2O and H_2O. Preliminary electrophysiological studies show that thioibotenic acid (**1**) is a moderately potent agonist (EC_{50} = 42 ± 2 μM) at the glutamic acid receptors sensitive to the *N*-methyl-D-aspartic acid (NMDA) receptor antagonist 4-(3-phosphonopropyl)-2-piperazinylcarboxylic acid (CPP).

(14) Purification of **7g** proved to be very difficult presumably due to the presence of impurities originating from polymerization of imine **6**.

In conclusion, we have developed a methodology for regioselective generation of the 5-lithio anion of the 3-oxygenated isothiazole heterocycle. To our knowledge, this is the first report on direct functionalization on this parental system. The 5-lithio anion of **4** showed high stability in diethyl ether, whereas in THF decomposition was progressing over time. A mechanism of decomposition was suggested based on identification of a degradation product. We were able to demonstrate the capture of the 5-lithio anion of **4** with a variety of electrophiles, which gave the desired products **7a**,**b**,**d**−**g** in 54−68% isolated yield, whereas attempted benzoylation to give **7c** was unsuccessful. The intermediate **7g** was deprotected in two steps to give thioibotenic acid (**1**), which will undergo further pharmacological characterization as a glutamic acid receptor ligand in our laboratory, and results will be reported elsewhere.

Experimental Section

All reagents were obtained from commercial suppliers and used without further purification. THF was distilled from sodium/benzophenone and Et_2O was dried over sodium. NMR (300 MHz) spectra were recorded in $CDCl_3$ using $CHCl_3$ as reference. Merck Kieselgel (35−70 mesh) was used for flash chromatography.

3-(Benzyloxy)isothiazole (4). To a solution of 3-hydroxyisothiazole[8] (2.02 g, 20 mmol) in DMF (20 mL) at 0 °C was added K_2CO_3 (5.53 g, 40 mmol) followed by benzyl bromide (2.74 mL, 23 mmol). The reaction mixture was allowed to stir for 48 h at rt, diluted with H_2O (200 mL), and extracted with Et_2O. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to give a crude product (2:1 O/N mixture based on ^{1}H NMR) that was purified by short column flash chromatography (heptane/EtOAc 4:1) followed by submission to high vacuum for 72 h to give **4** as a clear oil (2.29 g, 60%): ^{1}H NMR δ 8.46 (d, 1H, J = 5 Hz), 7.49−7.30 (m, 5H), 6.65 (d, 1H, J = 5 Hz), 5.42 (s, 2H); ^{13}C NMR δ 169.61, 149.00, 136.66, 128.65, 128.29, 128.25, 112.05, 70.49. Anal. Calcd for $C_{10}H_9NOS$: C, 62.80; H, 4.74; N, 7.32. Found: C, 63.05; H, 4.45; N, 7.17.

General Procedure for the Preparation of 5-Substituted 3-(Benzyloxy)isothiazoles (7a,b,d−g). To freshly prepared LDA (0.58 mmol) in Et_2O (4.5 mL) at −78 °C under N_2 was added dropwise 3-(benzyloxy)isothiazole (**4**) (100 mg, 0.52 mmol) dissolved in Et_2O (0.3 mL). After 15 min, the appropriate electrophile (0.58 mmol) dissolved in Et_2O (0.2 mL) was added and stirring continued at −78 °C for 15 min. The reaction mixture was quenched with saturated NH_4Cl and extracted with EtOAc. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated to give the crude products **7a**,**b**,**d**−**g**.

3-Benzyloxy-5-(α-hydroxybenzyl)isothiazole (7b). The reaction was carried out as described in the general procedure. The crude product was purified using flash chromatography (heptane/EtOAc 4:1, R_f = 0.25) to give **7b** as a white solid (102 mg, 65%): ^{1}H NMR δ 7.45−7.30 (m, 10H), 6.37 (s, 1H), 6.02 (s, 1H), 5.34 (s, 2H); ^{13}C NMR δ 172.70, 168.60, 141.90, 128.97, 128.79, 128.60, 128.25, 128.18, 126.52, 109.49, 71.88, 70,11. Anal. Calcd for $C_{17}H_{15}NO_2S$: C, 68.66; H, 5.08; N, 4.71. Found: C, 68.46; H, 4.83; N, 4.68.

3-Benzyloxy-5-(methoxycarbonyl)isothiazole (7d). The reaction was carried out as described in the general procedure. The crude product was purified using flash chromatography (heptane/EtOAc 9:1, R_f = 0.25) to give **7d** as a clear oil (73 mg, 56%): ^{1}H NMR δ 7.50−7.30 (m, 5H), 7.16 (s, 1H), 5.41 (s, 2H), 3.91 (s, 3H); ^{13}C NMR δ 168.59, 160.53, 156.36, 136.13, 128.69, 128.40, 128.22, 115.98, 70.61, 52.66. Anal. Calcd for $C_{12}H_{11}NO_3S$: C, 57.82; H, 4.45; N, 5.62. Found: C, 58.20; H, 4.49; N, 5.64.

3-Benzyloxy-5-formylisothiazole (7e). The reaction was carried out as described in the general procedure. The crude product was purified on flash chromatography (heptane/EtOAc 9:1, R_f = 0.2) to give **7e** as a light yellow solid (62 mg, 54%): ^{1}H NMR δ 10.00 (s, 1H), 7.50−7.35 (m, 5H), 7.14 (s, 1H), 5.43 (s, 2H); ^{13}C NMR δ 181.84, 169.06, 163.71, 135.93, 128.70, 128.53, 128.33, 117.09, 70.92. Anal. Calcd for $C_{11}H_9NO_2S$: C, 60.26; H, 4.14; N, 6.39. Found: C, 60.03; H, 3.97; N, 6.32.

3-Benzyloxy-5-(1-hydroxycyclohexyl)isothiazole (7f). The reaction was carried out as described in the general procedure. The crude product was purified using flash chromatography (heptane/EtOAc 4:1, R_f = 0.25) to give **7f** as a clear oil (102 mg, 68%): ^{1}H NMR δ 7.47−7.30 (m, 5H), 6.48 (s, 1H), 5.36 (s, 2H), 2.19 (s, 1H), 1.98−1.58 (m, 10H); ^{13}C NMR δ 179.62, 168.59, 136.65, 128.58, 128.18, 107.44, 72.59, 69.94, 39.34, 24.94, 21.63. Anal. Calcd for $C_{16}H_{19}NO_2S$: C, 66.40; H, 6.62; N, 4.84. Found: C, 66.28; H, 6.75; N, 4.65.

Ethyl 2-*tert*-Butyloxycarbonylamino-2-ethoxycarbonyl-2-(3-benzyloxy-5-isothiazolyl)acetate (7g). The reaction was carried out as described in the general procedure using imine **6** as the electrophile.[7] Isolation by flash chromatography (heptane/EtOAc 4:1, R_f = 0.25) gave crude **7g** as a clear oil (135 mg, 56%), which was not purified further: ^{1}H NMR δ 7.47−7.30 (m, 5H), 6.75 (s, 1H), 6.50 (br s, $^3/_4$ H), 6.30 (br s, $^1/_4$ H), 5.35 (s, 2H), 4.40−4.20 (m, 4H), 1.43 (br s, 9H), 1.26 (t, 6H, J = 7 Hz).

2-Amino-2-(3-hydroxy-5-isothiazolyl)acetic Acid (1). To a solution of **7g** (1.87 g, 4.02 mmol) in THF (50 mL) at rt was added LiOH (aq) (50 mL, 2.5 M) and the mixture stirred for 4 h. The reaction mixture was then cooled to 0 °C and the pH adjusted to 2 with 1 M HCl (aq). The aqueous phase was extracted with EtOAc, and the collective organic layers were washed with brine, dried (Na_2SO_4), and evaporated to give the crude product. Isolation by flash chromatography (CH_2Cl_2, MeOH, AcOH 100:5:2, R_f = 0.28) gave crude **8** as a clear oil (1.25 g, 76%) that was used without further purification. To a solution of **8** (1.25 g, 3.06 mmol) in glacial AcOH (15 mL) was added 10% HBr/AcOH (15 mL), and the reaction mixture was stirred at rt for 2 h. After evaporation, the crude product was trituated with Et_2O, H_2O (20 mL) was added, and the crystals that formed were filtered off. The crystals were washed with H_2O and then dried to give **1** as a yellow semicrystalline solid (360 mg, 60%):. ^{1}H NMR (DMSO) δ 9.05 (br s, 3H), 6.84 (s, 1H), 5.62 (br s, 1H); ^{13}C NMR (DMSO/$^-$OH) δ 178.84, 176.08, 167.05, 127.45, 113.19. Recrystallization of a sample from H_2O: mp = 178−180 °C. Anal. Calcd for $C_5H_6N_2O_3S$: C, 34.48; H, 3.47; N, 16.08. Found: C, 34.45; H, 3.32; N, 15.80.

Acknowledgment. We would like to thank Dr. Tine Bryan Steensbøl at the Royal Danish School of Pharmacy for the preliminary biological testing.

JO0162134