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# A new synthesis of dehydroluciferin [2-(6′-hydroxy-2′-benzothiazolyl)-thiazole-4-carboxylic acid] from 1,4-benzoquinone

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ABSTRACT

A new synthesis of dehydroluciferin [2-(6′-hydroxy-2′-benzothiazolyl)-thiazole-4-carboxylic acid], the oxidative product of luciferin, has been realized starting from 1,4-benzoquinone. Reaction of this compound with L-cysteine ethyl ester, followed by an oxidation–cyclization step afforded 2-carbethoxy-6-hydroxybenzothiazole that was in situ hydrolyzed and decarboxylated to 6-hydroxybenzothiazole. The *tert*-butyl(dimethyl)silyl ether of this key intermediate was subjected to α-lithiation followed by formylation with DMF, and the resulting aldehyde condensed with L-cysteine ethyl ester. Dehydrogenation of the intermediate thiazolidine followed by deprotection afforded dehydroluciferin in 35% overall yield from 1,4-benzoquinone (69% from 6-hydroxybenzothiazole).



## 1. Introduction

The luciferase from the North American firefly *Photinus pyralis* (PpyLuc; EC 1.13.12.7) catalyzes a bioluminescent reaction from D-luciferin {(*S*)-2-[6′-hydroxy-2′-benzothiazolyl]-2-thiazoline-4-carboxylic acid; **1**}, ATP, and oxygen. This reaction involves the formation of an enzyme-bound adenylyl intermediate (D-luciferyl adenylate; **2**) that is oxidized by molecular oxygen with release of pyrophosphate (PPi) and $CO_2$. The intermediate, unstable dioxetanone (**3**) liberates oxyluciferin {2-[6′-hydroxy-2′-benzothiazolyl]-4-hydroxythiazole, **4**} in an excited state that is stabilized by the emission of a photon (Scheme 1).[1,2] About 80% of the enzyme-bound intermediate D-luciferyl adenylate (**2**) is oxidized to oxyluciferin **4**, but another enzyme pathway gives rise to a side reaction in which the adenylate **2** is oxidized to the dehydroluciferyl adenylate (**5**) with production of $H_2O_2$. Pyrophosphorolysis of the adenylate **5** may occur with formation of dehydroluciferin [2-(6′-hydroxy-2′-benzothiazolyl)-thiazole-4-carboxylic acid, **6**].[3]

PpyLuc is a well characterized enzyme that finds a large number of biotechnological applications[4] and has been used, for example, as an indicator of cell proliferation, gene delivery or gene expression in cell culture and in living animals as a transgenic marker.[5] PpyLuc is at present the preferred enzyme for in vivo optical imaging of small animals.[6] A few limits such as light absorption and scattering by organs or the high dose of the reporter probe required for a sufficient emission[7] could be overcome by other imaging modalities, such as single photon emission computed tomography (SPECT) and positron emission tomography (PET), that use radionuclides for imaging of reporter genes.[8] In this respect, only two examples of radioactive isotopomers of D-luciferin (**1**) are available, i.e., 6′-[$^{11}$*C*-methyl]-D-luciferin[9] and 7′-[$^{123}$I]iodo-D-luciferin,[10] but these compounds were unable to locate the tumour and showed poor cell uptake.[9,11]

We have recently undertaken a project[12] aimed at developing compounds suitably labelled with radioisotopes for positron emission tomography (PET) in vivo imaging of a transgenic mouse that expresses a luciferase reporter gene under the control of activated oestrogen receptors.[13] We have considered the structure of PpyLuc inhibitors as possible targets for the introduction of the positron emitting isotope $^{18}$F.[14] Within this framework, we have recently prepared a few 2,6-disubstituted benzothiazoles[15] that have shown values of $IC_{50}$ (8.8–45.2 μM) that are in the range of other compounds of similar structure proposed as competitive inhibitors of PpyLuc ($K_i$ ranging from 25 to 58 μM).[16]

In this context, it is noteworthy that dehydroluciferin (**6**) itself is a good inhibitor of PpyLuc ($K_i$ 1 μM)[17] and that the most potent inhibitor of luciferase, DLSA [5′-*O*-[(*N*-dehydroluciferyl)-sulfamoyl]-adenosine, **7a** ($K_i$=340 nM)],[18] is the sulfamoyl analogue of dehydroluciferyl adenylate (**5**) (Fig. 1).

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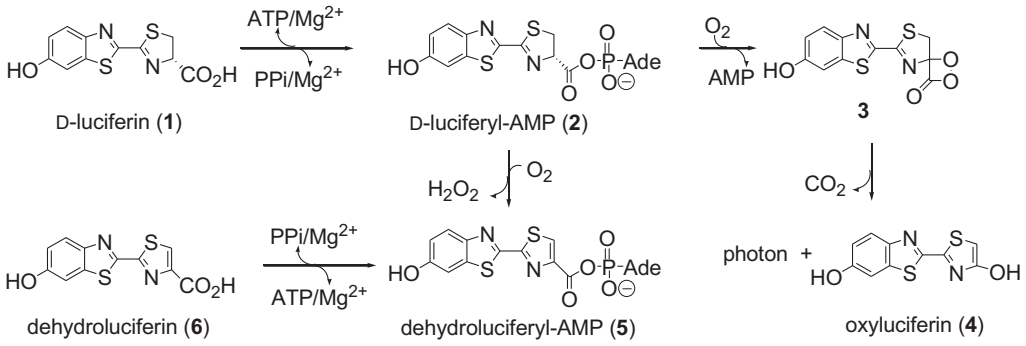



**Scheme 1.** PpyLuc-catalyzed reactions.

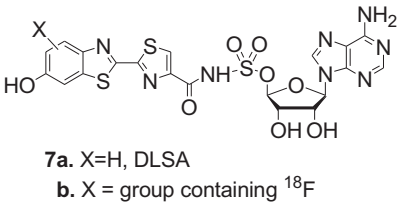
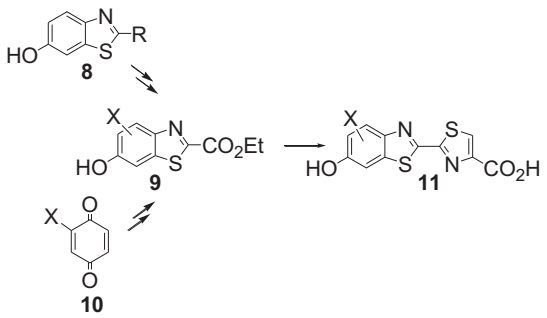



**Fig. 1.** 5′-*O*-[(*N*-Dehydroluciferyl)-sulfamoyl]-adenosine (DLSA, **7a**).

With the aim of preparing a $^{18}$F-labelled DLSA (compound **7b**), a group X should be placed in DLSA **7a** or in dehydroluciferin (**6**), its synthetic precursor. The group X should be transformed into a leaving group suitable to a nucleophilic substitution by $^{18}$F-fluoride, thus allowing the introduction of the $^{18}$F positron emitting isotope (compound **7b**). In a radiosynthesis, this step usually is accomplished following the experimental procedure traditionally established for the production of 2-[$^{18}$F]-fluoro-2-deoxy-D-glucose ($^{18}$F-FDG).[19]

For this project, two synthetic approaches to derivatives of dehydroluciferin substituted by a group X in the phenyl ring (compound **11**, Scheme 2) were considered. The group X could be introduced by an electrophilic substitution at the phenol moiety of a suitable 2,6-disubstituted benzothiazole (**8**) or dehydroluciferin (**6**) itself. Alternatively, the group X or a related moiety should be present in a synthetic precursor such as the substituted 1,4-benzoquinone **10**.

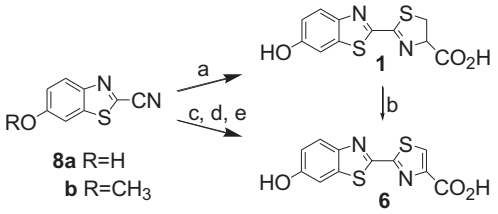



**Scheme 2.** Synthetic approaches to a dehydroluciferin derivative **11**.

In connection with our works on the synthesis of 2-substituted-6-hydroxy (or 6-methoxy) benzothiazoles[20] and D-luciferin (**1**) starting from 1,4-benzoquinone,[21] we have now developed a new synthesis of dehydroluciferin (**6**) starting from 1,4-benzoquinone.

## 2. Results and discussion

The synthesis of dehydroluciferin (**6**) has been described in the studies on the structure of D-luciferin (**1**) and structurally related compounds.[1,22] The original synthetic approach is reported in Scheme 3 and has been discussed in a recent review that summarizes syntheses of D-luciferin (**1**), derivatives and analogues.[23] The above preparations were carried out only on milligram scale and generally proceeded with low yields of the final products, since the intermediates 2-cyano-6-hydroxybenzothiazole (**8a**) and the 6-methoxy analogue **8b** were synthesized from *para*-anisidine with overall yields of 6 and 10%, respectively. The reaction of the nitrile **8a** with D-cysteine affords D-luciferin (**1**) that can be oxidized to dehydroluciferin (**6**) in a basic solution with either potassium ferricyanide or oxygen (61% from **8a**). Alternatively, dehydroluciferin (**6**) was prepared from 2-cyano-6-methoxybenzothiazole (**8b**) (16% yield).

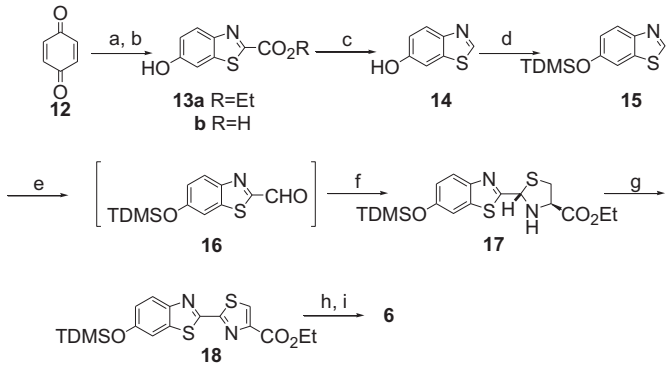



**Scheme 3.** Synthesis of dehydroluciferin (**6**) from 2-cyano-6-hydroxybenzothiazole (**8a**) or from 2-cyano-6-methoxybenzotiazole (**8b**). Reagents and conditions: (a) D-cystine/$NH_3$, Na, $H_2O$/MeOH 94%; (b) NaOH, $O_2$, 65%; (c) $H_2S$/$Et_3N$, Py, rt, 3 h, 95%; (d) methylbromopyruvate, MeOH, rt, 22 h and reflux 17 h, 88%; (e) HBr reflux, 1.5 h, 20%.

The initial steps of the synthesis of dehydroluciferin (**6**) from 1,4-benzoquinone (**12**) were carried out essentially as described.[20,21,24] Specifically, 1,4-benzoquinone **12** was converted into 2-carbethoxy-6-hydroxybenzothiazole **13a** that was not purified, but directly hydrolyzed in a basic medium. Acidification and decarboxylation of the unstable acid **13b** proceeded according to Löwik et al.[24] Purified 6-hydroxybenzothiazole **14** has been obtained by this simplified one-pot reaction in 50% yield and constitutes the starting material for our synthesis of dehydroluciferin (**6**) (Scheme 4).

The hydroxyl group of 6-hydroxybenzothiazole **14** was protected as the corresponding silyl ether and compound **15** was converted into the aldehyde **16** by reaction with butyllithium, followed by formylation with DMF. This procedure has been used to prepare heteroaromatic aldehydes regioselectively and in good yields.[25] For instance, the reaction has been recently applied to the preparation of 6-dimethylaminobenzothiazole-2-carbaldehyde.[26]

The aldehyde **16** was obtained in 89% yield from compound **15** as confirmed by the $^1$H NMR spectrum of the *crude* material,[27] and was directly reacted with L-cysteine methyl ester in refluxing hexane, smoothly affording the thiazolidine derivative **17** (84%). This compound has been obtained as an inseparable 3:2 mixture of C-2 epimers that show well-resolved signals in the 500 MHz $^1$H NMR spectrum.




**Scheme 4.** Synthesis of dehydroluciferin (**6**) from 1,4-benzoquinone (**12**). Reagents and conditions: (a) $CysCO_2Et$/MeOH; (b) $K_3Fe(CN)_6$/NaOH/*i*-PrOH; (c) NaOH (overall yield of steps a–c 50%); *t*-BuDSiCl/imidazole/THF, 98%; (e) BuLi/DMF/THF (not isolated); (f) $CysCO_2Et$/TEA/hexane (steps e and f 84%); (g) $MnO_2$/toluene, 95%; (h) TBAF/THF; (i) NaOH (steps h and i 88%).

Oxidation of the thiazolidine **17** to compound **18** was most conveniently performed with activated manganese dioxide $MnO_2$ in toluene in nearly quantitative yield. The doubly protected dehydroluciferin (compound **18**) was desilylated with tetrabutylammonium fluoride (TBAF) and then hydrolyzed with 4 M NaOH to give dehydroluciferin (**6**) (88% yield after the two deprotection steps). Yields of dehydroluciferin (**6**) were 35% from 1,4-benzoquinone (**12**) and 69% from 6-hydroxybenzothiazole (**14**).

## 3. Conclusions

In summary, starting from easily available 1,4-benzoquinone (**12**), a simple and convenient procedure has been developed to synthesize dehydroluciferin (**6**) in good yields (35% overall yield from benzoquinone). The new synthetic procedure here established allows for the first time the preparation of grams of dehydroluciferin (**6**), and is versatile enough to prepare substituted dehydroluciferins (compounds **11**). In fact, depending on the substituted 1,4-benzoquinone derivatives **10** (Scheme 3) either commercially available or ad hoc synthesized, a wide variety of groups X in the phenyl ring of dehydroluciferin (**6**) could be introduced.

This would open the access to $^{18}$F-labelled DLSA **7b** for PET in vivo imaging of the transgenic mouse that expresses a luciferase reporter gene under the control of activated oestrogen receptors.[13]

## 4. Experimental section

### 4.1. General

All of the reagents and solvents, analytically pure, were obtained from Sigma–Aldrich and used as such without further purification. Column chromatography was performed on Silica Gel 60 (70–230 mesh) using the specified eluents. The progress of the reactions was monitored by analytical thin-layer chromatography (TLC) on precoated glass plates (silica gel 60 $F_{254}$-plate-Merck, Darmstadt, Germany) and the products were visualized by UV light.

Purity of all products (≥99%) was verified by thin-layer chromatography and NMR measurements. Elemental analyses were obtained for all intermediates and are within ±0.4% of theoretical values.

Melting points were determined with a Stuart Scientific SMP3 melting point apparatus.

$^1$H NMR spectra were recorded at 298 K in $CDCl_3$ (isotopic enrichment 99.95%) or DMSO-$d_6$ (isotopic enrichment 99.98%) solutions at 300 K using a Bruker AVANCE 500 instrument (500.13 MHz for $^1$H, 125.76 MHz for $^{13}$C) using 5 mm inverse detection broadband probes and deuterium lock. Chemical shifts ($\delta$) are given in parts per million (ppm) and were referenced using residual signals of the solvent as internal standard ($^1$H: $CHCl_3$, 7.26 ppm; DMSO, 2.49 ppm. $^{13}$C: $CDCl_3$, 77.0 ppm; DMSO-$d_6$, 39.5 ppm). Coupling constants ($J$) are in hertz (Hz) and the experimental error in the measured $^1$H–$^1$H coupling constants is ±0.5 Hz. The splitting pattern abbreviations are as follows: s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet, and br s, broad peak. For two-dimensional experiments, Bruker microprograms using gradient selection (gs) were applied. Infrared spectra were recorded using a Jasco FTIR 4100 spectrometer as a thin film on NaCl plates.

### 4.2. 6-Hydroxybenzothiazole (14)

The previously described procedures[21,24] have been adapted to a one-pot preparation that is described here in detail. A solution of 1,4-benzoquinone (**12**) (1.0 g, 9.2 mmol) in methanol (20 mL) was slowly added to a solution of L-cysteine ethyl ester hydrochloride (1.7 g, 9.2 mmol) in methanol (10 mL) with stirring and cooling in an ice bath under nitrogen. After stirring at room temperature for 1.5 h, the reaction was monitored by TLC (petroleum ether/ethyl acetate 6:4) and the solvent was evaporated under vacuum. Isopropanol was added (60 mL) and the solution was cooled in an ice bath under nitrogen and stirring. An aqueous solution of 1 M $K_3Fe(CN)_6$ (36 mL) and 4 M NaOH (2.5 mL) was slowly added and stirring was continued at room temperature. At the end of the reaction (1 h) after TLC (petroleum ether/ethyl acetate, 8:2), the solid was removed by filtration under vacuum and the solution extracted with ethyl acetate. A small part of this solution was evaporated to dryness to get the crude product verifying the presence of desired compound 2-carbethoxy-6-hydroxybenzothiazole **13a**: $^1$H NMR ($CD_3OD$): $\delta$=7.97 (d, $J$=9.0 Hz, 1H, 4-H), 7.37 (d, $J$=2.0 Hz, 1H, 7-H), 7.13 (dd, $J$=9.0 and 2.0 Hz, 1H, 5-H), 4.50 (q, $J$=6.9 Hz, 2H, $CH_2CH_3$), 1.46 (t, $J$=6.9 Hz, 3H, $CH_2CH_3$) ppm.

The ethyl acetate extract was then washed with 1 M NaOH (50 mL), the aqueous phase was acidified with 3 M HCl, and extracted with ethyl acetate. Evaporation of the solvent at 60 °C caused complete decarboxylation of the unstable acid **13b**. Purification by column chromatography (DCM/acetone 95:5) gave title compound **14** as a colourless solid that was recrystallized from aqueous ethanol (0.7 g, 4.6 mmol, 50%). Mp 190–192 °C (lit.[24] 184–185 °C). $R_f$=0.35 (petroleum ether/AcOEt 6:4). $^1$H NMR ($CD_3OD$): $\delta$=8.98 (s, 1H, 2-H), 7.88 (d, $J$=8.3 Hz, 1H, 4-H), 7.38 (d, $J$=2.1 Hz, 1H, 7-H), 7.06 (dd, $J$=8.3 and 2.1 Hz, 1H, 5-H) ppm. $^{13}$C NMR ($CD_3OD$): $\delta$=164.8 (6-C), 155.9 (9-C), 152.1 (2-CH), 134.9 (8-C), 122.8 (4-CH), 115.9 (5-CH), 106.1 (7-CH) ppm.

### 4.3. 6-{[*tert*-Butyl(dimethyl)silyl]oxy}-1,3-benzothiazole (15)

Imidazole (400 mg) and *tert*-butyldimethylsilyl chloride (800 mg) were added to a solution of **14** (0.7 g, 4.6 mmol) in dry tetrahydrofuran (15 mL) under stirring and nitrogen at room temperature. The reaction was monitored by TLC (petroleum ether/ethyl acetate, 8:2) until all the starting material was consumed (approximately 18 h). A solution of ammonium chloride was added and the mixture was extracted with ethyl acetate. Purification by column chromatography (petroleum ether/ethyl acetate 8:2) gave title compound **15** as a colourless oil (1.2 g, 4.5 mmol, 98%). $R_f$=0.38 (petroleum ether/AcOEt 9:1). $^1$H NMR ($CDCl_3$): $\delta$=8.85 (s, 1H, 2-H), 7.99 (d, $J$=9.0 Hz, 1H, 8-H), 7.38 (d, $J$=2.0 Hz, 1H, 5-H), 7.05 (dd, $J$=9.0 and 2.0 Hz, 1H, 7-H), 1.03 (s, 9H, $SiC(CH_3)_3$), 0.32 (s, 6H, $2\times Si(CH_3)_2$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$=153.9 (6-C), 151.8 (2-CH), 148.3 (9-C), 134.9 (4-C), 123.9 (8-CH), 120.2 (7-CH), 111.7 (5-CH), 25.7 ($3\times Si-CH_3$), 18.3 (Si–C), −4.4 ($2\times Si-CH_3$) ppm; IR (NaCl, neat) 3063, 2930, 2857, 2711, 2360, 1898, 1553, 1445, 1390, 1268, 1186,

1046, 947, 854, 815, 738, 686, 613 $cm^{-1}$; $C_{13}H_{19}NOSSi$ (265.45): C 58.82, H 7.21, N 5.28, S 12.08; found C 58.89, H 7.16, N 5.20, S 12.14.

### 4.4. Ethyl 2-{6′-[*tert*-butyl(dimethyl)silyl]oxy-2′-benzothiazolyl}-thiazolidine-4-carboxylate (17)

A solution of *n*-butyllithium (3 mL 1.6 M in hexane, 4.8 mmol) was added dropwise to a stirred solution of **15** (1.2 g, 4.5 mmol) in THF (27 mL) at −78 °C at a rate that kept the reaction temperature below −70 °C. On completion of addition, the dark-red reaction mixture was stirred for 30 min at −78 °C and a solution of DMF (0.35 mL, 4.5 mmol) in tetrahydrofuran (1 mL) was added. After 1.5 h, the reaction mixture was allowed to warm to room temperature and was quenched with saturated aqueous $NH_4Cl$ (10 mL). The layers were separated, and the aqueous layer was extracted with $Et_2O$. The combined organic extracts were dried ($Na_2SO_4$) and concentrated in vacuo to give 6-{[*tert*-butyl(dimethyl)silyl]oxy}-1,3-benzothiazol-2-carbaldehyde (**16**) as an oil, immediately used for the next reaction. $R_f$=0.62 (petroleum ether/AcOEt 9:1). $^1$H NMR ($CDCl_3$): $\delta$=10.1 (s, 1H, CHO), 8.10 (d, $J$=8.3 Hz, 1H, 4-H), 7.37 (d, $J$=2.1 Hz, 1H, 7-H), 7.15 (dd, $J$=8.3 and 2.1 Hz, 1H, 5-H), 1.03 (s, 9H, $SiC(CH_3)_3$), 0.28 (s, 6H, 2×$Si(CH_3)_2$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$=185.2 (CHO), 162.7 (2-CH), 156.7 (6-C), 148.7 (9-C), 138.3 (8-C), 126.7 (4-CH), 122.1 (5-CH), 111.8 (7-CH), 25.7 (3×Si−C$(CH_3)_3$), 18.3 (Si−C), −4.34 (2×Si−$CH_3$) ppm.

A mixture of aldehyde **16**, L-cysteine ethyl ester hydrochloride (920 mg, 5 mmol), and triethylamine (1.2 mL, 8.6 mmol) in hexane (30 mL) was refluxed under stirring for 2 h. At the end of the reaction after TLC (petroleum ether/ethyl acetate 8:2), the solid was removed by filtration under vacuum and the solution evaporated to provide **17** (3:2 mixture of C-2 epimers) as an oil (1.6 g, 3.8 mmol, 84%). $R_f$=0.48 (petroleum ether/AcOEt 8:2). $^1$H NMR ($CDCl_3$): $\delta$=(major epimer) 7.86 (d, $J$=8.5 Hz, 1H, 4′-H), 7.28 (d, $J$=2.1 Hz, 1H, 7′-H), 6.99 (dd, $J$=8.5 and 2.1 Hz, 1H, 5′-H), 5.87 (s, 1H, 5-H), 4.31–4.25 (m, 3H, 4-H and O$CH_2CH_3$), 3.44 (dd, $J$=10.5 and 6.6 Hz, 1H, 5a-H), 3.17 ( dd, $J$=10.5 and 9.4 Hz, 1H, 5b-H), 1.33 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.00 (s, 9H, $SiC(CH_3)_3$), 0.22 (s, 6H, 2×$Si(CH_3)_2$) ppm. $^{13}$C NMR ($CDCl_3$): 171.3 ($CO_2$Et), 170.4 (2′-C), 153.6 (6′-C), 148.7 (9′-C), 136.4 (8′-C), 123.6 (4′-CH), 120.0 (5′-CH), 111.7 (7′-CH), 67.0 (2-C), 64.7 (4-C), 62.0 ($CO_2CH_2CH_3$), 38.0 (5-CH), 25.7 (3×Si−C$(CH_3)_3$), 18.3 (Si−C), 14,2 ($CO_2CH_2CH_3$), −4.3 (2×Si−$CH_3$) ppm.

$\delta$=(minor epimer) 7.82 (d, $J$=8.5 Hz, 1H, 4′-H), 7.25 (d, $J$=2.1 Hz, 1H, 7′-H), 6.97 (dd, $J$=8.5 and 2.1 Hz, 1H, 5′-H), 6.08 (s, 1H, 5-H), 4.31–4.25 (m, overlapped, 2H, O$CH_2CH_3$), 4.05 (dd, $J$=9.4 and 6.6 Hz, 1H, 4-H), 3.45 (dd, $J$=10.5 and 6.6 Hz, 1H, 5a-H), 3.15 (dd, $J$=10.5 and 9.4 Hz, 1H, 5b-H), 1.32 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 0.99 (s, 9H, $SiC(CH_3)_3$), 0.21 (s, 6H, 2×$Si(CH_3)_2$) ppm. $^{13}$C NMR ($CDCl_3$): 172.8 ($CO_2$Et), 166.8 (2′-C), 153.9 (6′-C), 147.8 (9′-C), 136.3 (8′-C), 123.9 (4′-CH), 120.3 (5′-CH), 111.7 (7′-CH), 68.0 (2-C), 66.1 (4-C), 61.8 ($CO_2CH_2CH_3$), 38.7 (5-CH), 25.7 (3×Si−C$(CH_3)_3$), 18.3 (Si−C), 14,2 ($CO_2CH_2CH_3$), −4.3 (2×Si−$CH_3$) ppm; IR (NaCl, neat) 3942, 3330, 2930, 2983, 2931, 2858, 2341, 1737, 1554, 1454, 1391, 1265, 1096, 1030, 947, 896, 857, 825, 782, 704 $cm^{-1}$; $C_{19}H_{28}N_2O_3S_2Si$ (424.13): C 53.74, H 6.65, N 6.60, S 15.10; found C 53.80, H 6,66, N 6.72, S 15.18.

### 4.5. Ethyl 2-(6′-[*tert*-butyl(dimethyl)silyl]oxy-2′-benzothiazolyl)-thiazole-4-carboxylate (18)

To a solution of compound **17** (1.6 g, 3.8 mmol) in toluene (30 mL) activated $MnO_2$ (0.35 g, 4 mmol) was added. The reaction mixture was refluxed for 4 h. At the end of the reaction, after TLC (petroleum ether/ethyl acetate 6:4) the solid was removed by filtration through a Celite pad under vacuum and the solvent evaporated to give title compound **18** as a yellow solid (1.5 g, 3.6 mmol, 95%). Mp 138–140 °C. $R_f$=0.52 (petroleum ether/AcOEt 8:2). $^1$H NMR ($CDCl_3$): $\delta$=8.28 (s, 1H, 5-H), 7.94 (d, $J$=8.8 Hz, 1H, 4′-H), 7.35 (d, $J$=2.5 Hz, 1H, 7′-H), 7.04 (dd, $J$=8.8 and 2.5 Hz, 1H, 5′-H), 4.46 (q, $J$=7.0 Hz, 2H, O$CH_2CH_3$), 1.44 (t, $J$=7.0 Hz, 3H, $OCH_2CH_3$), 1.02 (s, 9H, $SiC(CH_3)_3$), 0.26 (s, 6H, 2×$Si(CH_3)_2$) ppm. $^{13}$C NMR ($CDCl_3$): $\delta$=162.5 (2-C) 160.9 ($CO_2$Et), 158.2 (6′-C), 154.9 (2′-C), 148.4 (9′-C), 148.2 (4-C), 137.2 (8′-C), 129.4 (5-CH), 124.1 (4′-CH), 121.0 (5′-CH), 111.8 (7′-CH), 61.8 ($CO_2CH_2CH_3$), 25.7 (3×Si−C$(CH_3)_3$), 18.3 (Si−C), 14.4 ($CO_2CH_2CH_3$), −4.3 (2×Si−$CH_3$) ppm; IR (NaCl, neat) 3053, 2930, 2957, 2896, 2360, 1730, 1551, 1494, 1391, 1336, 1265, 1212, 1101, 1022, 905, 822, 782, 704, 646 $cm^{-1}$; $C_{19}H_{24}N_2O_3S_2Si$ (420.62): C 54.25, H 5.75, N 6.66, S 15.25; found C 54.32, H 5.66, N 6.69, S 15.18.

### 4.6. 2-(6'-Hydroxy-2′-benzothiazolyl)-thiazole-4-carboxylic acid (6)

To a solution of compound **18** (1.5 g, 3.6 mmol) in THF (30 mL) a 1 M solution in THF of tetrabutylammonium fluoride (7 mL, 7 mmol) was added. After 15 min the reaction mixture was washed with a solution of sodium bicarbonate, extracted with DCM, and concentrated to a small volume. To the remained THF solution 4 M sodium hydroxide was added (2 mL, 8 mmol) and the mixture was stirred at room temperature for 3 h. At the completion of the reaction, 3 M HCl (3 mL, 9 mmol) was added. After filtration and washing with diethyl ether the product was recovered as a solid (0.89 g, 3.2 mmol, 88%). Mp 315 °C (lit.[22] 315–321 °C dec). $R_f$=0.13 (DCM/MeOH 8:2). $^1$H NMR (DMSO-$d_6$): $\delta$=10.16 (s, 1H, $CO_2H$), 8.64 (s, 1H, 5-H), 7.92 (d, $J$=8.8 Hz, 1H, 4′-H), 7.47 (d, $J$=2.4 Hz, 1H, 7′-H), 7.05 (dd, $J$=8.8 and 2.4 Hz, 1H, 5′-H), 10.16 (s, 1H, OH) ppm. $^{13}$C NMR (DMSO-$d_6$): $\delta$=162.1 ($CO_2H$), 161.8 (2-C) 157.5 (6′-C), 157.0 (2′-C), 148.8 (9′-C), 146.8 (4-C), 137.2 (8′-C), 131.6 (5-CH), 124.7 (4′-CH), 117.6 (5′-CH), 107.5 (7′-CH) ppm.

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27. The aldehyde **16** could be obtained also by a traditional reduction–oxidation of the ester **13a**, but overall yields were lower than the ones described in this paper.