Synthesis and Characterization of *o*-Carboranylthiolate Substituted 1,3,5-Triazines

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Mercaptoundecahydrododecaborate ($B_{12}H_{11}SH^{2-}$),[1] known as BSH, is a water-soluble divalent anionic boron cluster containing a SH subunit, exhibiting significantly low toxicity. For this reason, BSH has been successfully adopted as a potential agent in the area of boron neutron capture therapy (BNCT).[2] *o*-Carboranylthiol ($C_2B_{10}H_{11}SH$)[3] is similar to BSH in terms of having a SH functional unit in a boron cluster (Figure 1). Thiol functional group can be useful to anchor *o*-carboranyl group to the 1,3,5-triazine[4] unit. There are prototype reactions involving a nucleophilic triazine substitution[5] with organic N, O, and S-functional groups. Therefore, in this paper, *o*-carboranylthiolate was employed as a potential nucleophile to triazine network to generate *o*-carboranylthiolate functionalized-1,3,5-triazine derivatives such as 6-(*o*-carboranylthiolato)-2,4-bis[di(methoxyethyl)-amino]-1,3,5-triazine (**3**) and 4,6-bis(*o*-carboranylthiolato)-2-[di(methoxyethyl)amino]-1,3,5-triazine (**5-7**).

Several *o*-carboranylthiolates, derived from *o*-carborane and methyl- and phenyl-*o*-carborane, were utilized as nucleophiles to generate mono- and bis(*o*-carboranylthiolate) triazines. Chloro-1,3,5-triazines (**2**) were prepared according to the established synthetic protocols. Thus, **2** was obtained by reacting **1** with di(2-methoxyethyl)amine in 1:2 (Scheme 1). *o*-Carboranylthiolate was generated *in situ* by the reaction of *o*-carborane with an equimolar *n*-butyllithium, followed by addition of S_8 in anhydrous THF at −78 °C. Subsequent reaction with chloro-1,3,5-triazines (**2**) generated the mono-substituted *o*-carboranythiolato-1,3,5-triazine (**3**) in 14% yield (Scheme 1).

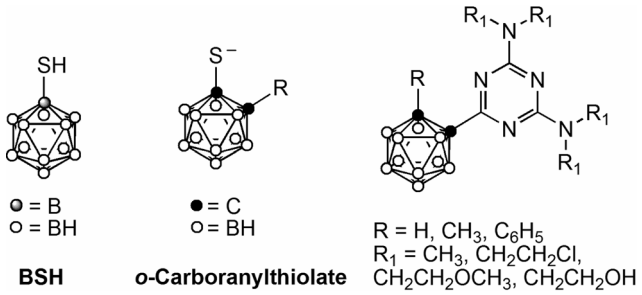
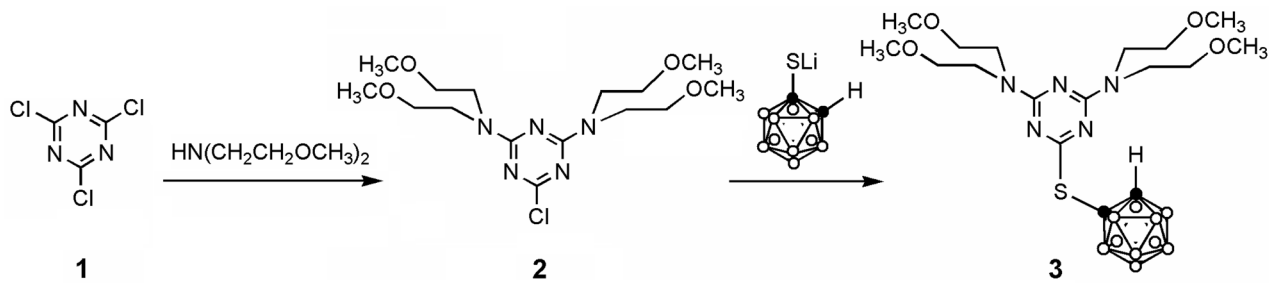


Figure 1. BSH, *o*-carboranylthiolate, and *o*-carboranyl-1,3,5-triazine derivatives.

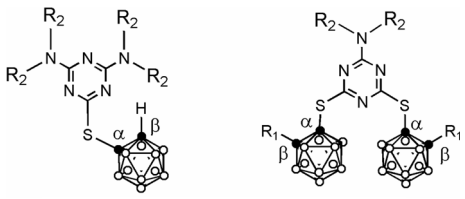
In the ^{1}H NMR spectrum of **3**, diagnostic signals were observed for the ethylene protons in NCH_2CH_2O at around δ 3.57-3.83. Key signals detected in the ^{13}C NMR spectrum of **3** include resonances at around δ 59.2-67.5 (NCH_2CH_2O), 62.9 (*C*-β), 70.4 (*C*-α), and 161.7-175.6 (triazine ring). A summary of selected physical and spectroscopic properties of **3** is presented in Table 1.

Dichloro-1,3,5-triazine (**4**) was prepared according to the established synthetic protocols. Thus, **4** was obtained by reacting **1** with di(2-methoxyethyl)amine in 1:1 stoichiometry (Scheme 2). When dichloro-1,3,5-triazine (**4**) was reacted with two equivalent amount of lithium *o*-carboranyl-dithiolates in THF at −78 °C, the compounds **5-7** were formed in 31-92% yields (Scheme 2). In the ^{1}H NMR spectra of bis(*o*-carboranylthiolato)-1,3,5-triazine derivatives (**5-7**), diagnostic signals were observed for the ethylene protons of NCH_2CH_2O at around δ 3.50-3.95. Key signals detected in the ^{13}C NMR spectra of **5-7** include resonances at around δ 59.1-79.6 (NCH_2CH_2O), 62.9-71.1 (*C*-β), 70.4-89.1 (*C*-α), and 161.7-175.6 (triazine ring). A summary of selected physical and spectroscopic properties of **5-7** is



Scheme 1. Preparation of 6-(*o*-carboranylthiolato)-2,4-bis[di(methoxyethyl)amino]-1,3,5-triazine (**3**).

Table 1. Summary of Selected Spectral Properties of the sulfur linked *o*-carboranylthiolato-1,3,5-triazine Derivatives **3**, **5**, **6**, and **7**



No.	Mp (°C)[a]	Yield (%)[b]	IR ν(B-H)	NMR (^{1}H/^{13}C)				
				NCH_2	OCH_2	Triazine	C(α)	C(β)
1	54-56	91		3.57	3.85			
				58.9	70.1	169.9, 164.8		
3	112-114	92	2586	3.50	3.77			5.00
				59.2	67.5	175.6, 161.7	70.4	62.9
5	94-96	14	2594	3.57	3.83			5.06
				59.2	67.5	175.6, 161.7	70.4	62.9
6	138-139	62	2590	3.61	3.95			
				59.1	75.3	174.8, 161.9	80.1	71.1
7	51-52	31	2592	3.64	3.95			
				59.1	79.6	174.8, 161.9	89.1	71.1

[a]Melting points are uncorrected. [b]Purified yields

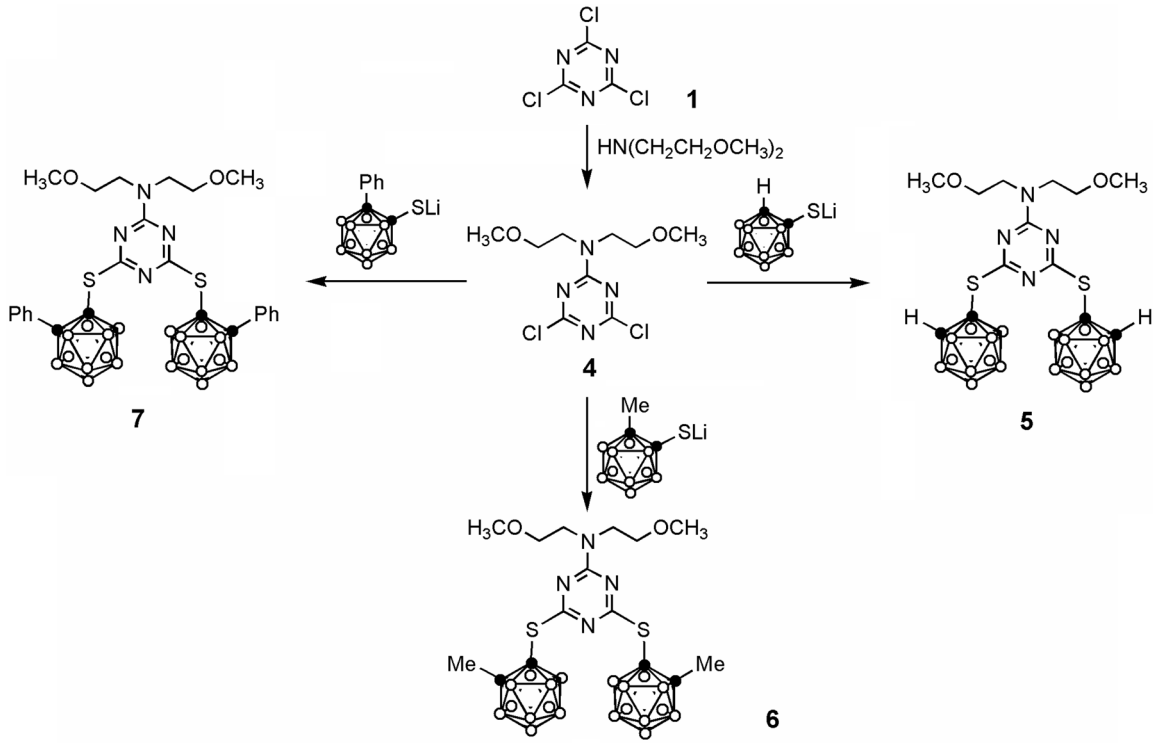
presented in Table 1. Bis(*o*-carboranylthiolate) substitution is manifested by the X-ray structural study (Figure 2). Crystallograpic data for the structure reported here have been deposited with the Cambridge Crystallographic Data Centre (Deposition No. CCDC-671564). That data can be obtained free of charge *via* http://www.ccdc.cam.ac.uk/perl/catreq.cgi.

Compounds **3**, **5**, **6**, and **7** are moderately stable in air and decomposed slowly when they are in contact with light and moisture. Contrary to our expectation, toxicity of *o*-carboranylthiolato-1,3,5-triazine derivatives (**3**, **5**, **6**, and **7**) was not improved. However, based on the results of *in vitro* studies, boron uptake in B-16 melanoma cells were found to be significantly increased (see Table 2).

Experimental Section

6-Chloro-2,4-bis[di(2-methoxyethyl)amino]-1,3,5-triazine (2). To a stirred solution of cyanuric chloride **1** (1.84 g, 10 mmol) and *N*,*N*-diisopropylethylamine (2.58 g, 20 mmol) in THF, which was cooled to −10 °C, was added di(2-methoxyethyl)amine (2.66 g, 20 mmol) *via* a syringe. After the reaction temperature was maintained at −10 °C for 1 h, the reaction mixture was warmed slowly to room temperature. After being stirred for additional 12 h, the reaction was quenched with distilled H_2O (50 mL). The crude product was extracted with diethyl ether (30 mL×2). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated *in vacuo* to yield 3.62 g (96%) of **2**. Mp. 94-95 °C. HRMS: m/z: calcd for $C_{15}H_{28}ClN_5O_4$: 377.1830; found: 377.1844 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 12H), 3.58 (t, J = 5.5 Hz, 8H), 3.86 (t, J = 5.5 Hz, 8H). ^{13}C NMR ($CDCl_3$) δ 48.6, 59.0, 70.1, 164.8, 169.9.

6-(*o*-Carboran-1-ylthiolato)-2,4-bis[di(2-methoxyethyl)-amino]-1,3,5-triazine (3). To a stirred solution containing



Scheme 2. Preparation of 4,6-bis(*o*-carboranylthiolato)-2-[di(methoxyethyl)amino]-1,3,5-triazine (**5**-**7**).

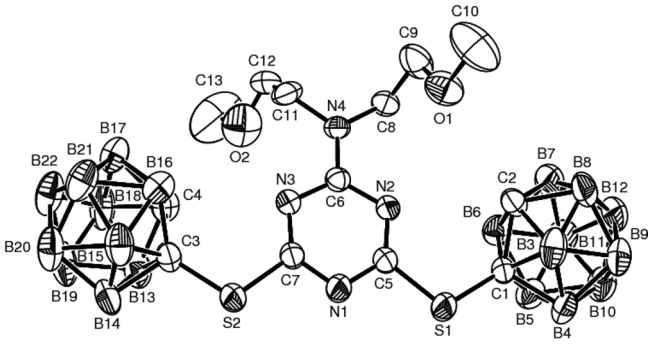


Figure 2. Molecular structure of compound **5**. The thermal ellipsoids are drawn at the 30% probability level.

Table 2. Cytotoxicity (IC_{50}) for B-16 and Boron Uptake

No.	Compound	IC_{50} (M)[a]	Boron Uptake[b] (μg B/10^6 cells)
1	**3**	6.32×10^{-5} ($\pm$0.65)	0.34 $\pm$ 0.049
2	**5**	>100	0.12 $\pm$ 0.0028
3	**BPA**	4.49×10^{-5} ($\pm$0.30)	0.12 $\pm$ 0.0028

[a]B-16: B-16 melanoma cell. [b]Boron uptake by B-16 cells was determined using the ICP-AES method.[8]

o-carborane (0.14 g, 1 mmol) was added *n*-BuLi (2.5 M, 0.4 mL, 1 mmol) in 20 mL of THF at −78 °C for 30 min, and then added sulfur (S_8) (0.032 g, 1 mmol). After 6 h, compound **2** (0.38 g, 1 mmol) was added to the reaction mixture. The mixture was stirred at −78 °C for 30 min, and then warmed to room temperature. After 12 h, this reaction was quenched with distilled H_2O (20 mL). The mixture was extracted with diethyl ether (20 mL × 2). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated *in vacuo*. The product **3** was isolated by flash column chromatography (EA:Hx 1:4) in a 14% yield (0.07 g). Mp. 94-96 °C. HRMS: m/z: calcd for $C_{17}H_{39}B_{10}N_5O_4S$: 519.3653; found: 519.3611 $[M+H]^+$. IR (KBr pellet, cm^{-1}) ν(B-H) 2594. ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 12H), 3.57 (t, J = 5.0 Hz, 8H), 3.83 (t, J = 5.0 Hz, 8H), 5.06 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 48.3, 59.2, 62.9, 67.5, 70.4, 161.7, 175.6.

4,6-Dichloro-2-[di(2-methoxyethyl)amino]-1,3,5-triazine (4). To a stirred solution of cyanuric chloride **1** (1.84 g, 10 mmol) and *N*,*N*-diisopropylethylamine (1.3 g, 10 mmol) in THF, which was cooled to −10 °C, was added di(2-methoxyethyl)amine (1.33 g, 10 mmol) via a syringe. After the reaction temperature was maintained at −10 °C for 1 h, the reaction mixture was warmed slowly to room temperature. After being stirred for additional 12 h, the reaction was quenched with distilled H_2O (50 mL). The crude product was extracted with diethyl ether (30 mL × 2). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated *in vacuo* to yield 2.56 g (91%) of **4**. Mp. 54-56 °C. HRMS: m/z: calcd for $C_9H_{14}Cl_2N_4O_2$: 280.0494; found: 280.0499 $[M+H]^+$. ^{1}H NMR ($CDCl_3$) δ 3.32 (s, 6H), 3.57 (t, J = 5.5 Hz, 4H), 3.85 (t, J = 5.5 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 48.6, 58.9, 70.1, 164.8, 169.9.

4,6-Bis(*o*-Carboran-1-ylthiolato)-2-[di(2-methoxyethyl)-amino]-1,3,5-triazine (5). To a stirred solution containing *o*-carborane (0.42 g, 3 mmol) was added *n*-BuLi (2.5 M, 1.2 mL) in 30 mL of THF at −10 °C for 45 min, and then added sulfur (S_8) (0.096 g, 3 mmol). After 12 h, compound **4** (0.42 g, 1.5 mmol) was added to the reaction mixture at −10 °C. The mixture was stirred at −10 °C for 30 min then warmed to room temperature. After 12 h, this reaction was quenched with distilled water (30 mL). The mixture was extracted with diethyl ether (30 mL × 2). The organic layer was washed with H_2O, dried with anhydrous Na_2SO_4, and concentrated *in vacuo*. The product **5** was isolated by flash column chromatography (EA:Hx 1:4) in a 92% yield (0.77 g). Mp. 112-114 °C. HRMS: m/z: calcd for $C_{13}H_{36}B_{20}N_4O_2S_2$: 564.4141; found: 564.4100 $[M+H]^+$. IR (KBr pellet, cm^{-1}) ν(B-H) 2586. ^{1}H NMR ($CDCl_3$) δ 3.26 (s, 6H), 3.50 (t, J = 5.0 Hz, 4H), 3.77 (t, J = 5.0 Hz, 4H), 5.00 (s, 2H). ^{13}C NMR ($CDCl_3$) δ 48.3, 59.2, 62.9, 67.5, 70.4, 161.7, 175.6.

6: Yield. 96% (0.85 g). Mp. 110-112 °C. HRMS: m/z: calcd for $C_{15}H_{40}B_{20}N_4O_2S_2$: 592.4454; found: 592.4497 $[M+H]^+$. IR (KBr pellet, cm^{-1}) ν(B-H) 2561. ^{1}H NMR ($CDCl_3$) δ 1.52 (s, 6H), 3.32 (s, 6H), 3.66 (t, J = 5.5 Hz, 4H), 3.80 (t, J = 5.5 Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 21.4, 49.2, 56.1, 59.5, 71.2, 73.7, 162.4, 167.7.

7: Yield. 98% (1.05 g). Mp. 105-107 °C. HRMS: m/z: calcd for $C_{25}H_{44}B_{20}N_4O_2S_2$: 716.4767; found: 716.4726 $[M+H]^+$. IR (KBr pellet, cm^{-1}) ν(B–H) 2558. ^{1}H NMR ($CDCl_3$) δ 3.38 (s, 6H), 3.54 (t, J = 5.5 Hz, 4H), 3.75 (t, J = 5.5 Hz, 4H), 6.85-7.33 (m, 10H). ^{13}C NMR ($CDCl_3$) δ 48.5, 54.2, 57.5, 68.2, 69.7, 158.2, 159.5, 160.7, 163.4, 168.7.

Crystallographic Data of Compound 5: $C_{13}H_{34}B_{20}N_4O_2S_2$, Monoclinic, $P2_1/n$, a = 14.095(7) Å, b = 12.821(7) Å, c = 18.244(9) Å, β = 107.27(1)°, V = 3148(3) Å^3, Z = 4, D = 1.179 g/cm^3, F(000) = 1152, R_1 = 0.0777, wR_2 = 0.2115. Preliminary examination and data collection were performed using a Bruker SMART CCD detector system single-crystal X-ray diffractometer equipped with a sealed-tube X-ray source (40 kV × 50 mA) using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å). Preliminary unit cell constants were determined with a set of 45 narrow-frame (0.3° in ω) scans. The double-pass method of scanning was used to exclude any noise. The collected frames were integrated using an orientation matrix determined from the narrow-frame scans. The SMART software package was used for data collection, and SAINT was used for frame integration.[6] Final cell constants were determined by a global refinement of *xyz* centroids of reflections harvested from the entire data set. Structure solution and refinement were carried out using the SHELXTL-PLUS software package.[7]

Determination of IC_{50}. The boron compounds (20 mg) was dissolved in 1.0 mL of DMSO, and the resulting solution was diluted with Eagle's MEM (10% FCS), or BPA (*p*-boronophenylalanine) was directly dissolved in the same medium. In Falcon 3072 96-well culture plate, the cells (1×10^3 cells/well) were cultured on five wells with the medium containing boron compounds at various concentrations (1-100 ppm), and incubated for 3 days at 37 °C in CO_2 incubator. It is known that DMSO is non-toxic at the

concentration lower than 0.5%. We also confirmed by the control experiment that DMSO was non-toxic at the concentrations shown above. The medium was removed, and the cells were washed three times with PBS (–) (phosphate-buffered saline) and then MTS Assay for counting cells on Microplate reader. The results are presented as the concentration of agents that resulted in 50% of the cell number of untreated cultures (IC_{50}).

***In vitro* Boron Incorporation into B-16 Melanoma Cells.** B-16 melanoma cells were cultured in Falcon 3025 dishes (90 mmϕ). When the cells were grown to fill up the dish (3.0×10^6 cells/dish), the boron compounds (1.0×10^{-4} M, 1.08 ppm boron) and BPA (1.0×10^{-3} M, 10.8 ppm boron) were added to dishes, The cells were incubated for 3 h at 37 °C in 20 mL of the medium (Eagle-MEM, 10% FBS). The cells were washed 3 times with Ca-Mg free phosphate buffered saline [PBS (–)], collected by rubber policeman, digested with 2 mL of 60% $HClO_4$-30% H_2O_2 (1:2) solution and then decomposed for 1 h at 75 °C. After filtration with membrane filter (Millipore, 0.22 μm), the boron concentration was determined by using ICP-AES (Shimadzu, ICPS-1000-III). Three replications of each experiment were carried out. The average of boron concentrations of each fraction was indicated in Table 2.

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References

1. (a) Elhanati, G.; Salomon, Y.; Bendel, P. *Cancer Lett*. **2001**, *172*, 127. (b) Awad, D.; Tabod, I.; Lutz, S.; Wessolowski, H.; Gabel, D. *J. Organomet. Chem*. **2005**, *690*, 2732.
2. (a) Nakamura, H.; Ueno, M.; Lee, J.-D.; Ban, H. S.; Justus, E.; Fan, P.; Gabel, D. *Tetrahedron Lett*. **2007**, *48*, 3151. (b) Sano, T. *Bioconjugate Chem*. **1999**, *10*, 905. (c) Azev, Y.; Lork, E.; Duelcks, T.; Gabel, D. *Tetrahedron Lett*. **2004**, *45*, 3249. (d) Gabel, D.; Awad, D.; Schaffran, T.; Radovan, D.; Dărăban, D.; Damian, L.; Winterhalter, M.; Karlsson, G.; Edwards, K. *ChemMedChem*. **2007**, *2*, 51. (e) Justus, E.; Awad, D.; Hohnholt, M.; Schaffran, T.; Edwards, K.; Karlsson, G.; Damian, L.; Gabel, D. *Bioconjugate Chem*. **2007**, *18*, 1287.
3. (a) Wang, J.; Zheng, C.; Maguire, J. A.; Hosmane, N. S. *Organometallics* **2003**, *22*, 4839. (b) Todd, J. A.; Caiazza, D.; Tiekink, E. R. T.; Rendina, L. M. *Inorg. Chim. Acta* **2003**, *352*, 208. (c) Laromaine, A.; Teixidor, F.; Kivekäs, R.; Sillanpää, R.; Benakki, R.; Grüner, B.; Viñas, C. *Dalton Trans*. **2005**, 1785.
4. (a) Azev, Y.; Slepukhina, I.; Gabel, D. *Appl. Radiation Isotopes* **2004**, *61*, 1107. (b) Woodhouse, S. L.; Ziolkowski, E. J.; Rendina, L. M. *Dalton Trans*. **2005**, 2827.
5. (a) Maheswari, P. U.; Modec, B.; Pevec, A.; Kozlevèar, B.; Massera, C.; Gamez, P.; Reedijk, J. *Inorg. Chem*. **2006**, *45*, 6637. (b) Milton, M. D.; Kumar, N.; Sokhi, S. S.; Singh, S.; Singh, J. D. *Tetrahedron Lett*. **2004**, *45*, 6453. (c) Milton, M. D.; Kumar, N.; Sokhi, S. S.; Singh, S.; Maheshwari, M.; Singh, J. D.; Asnani, M.; Butcher, R. J. *Tetrahedron Lett*. **2004**, *45*, 8941. (d) Shastin, A. V.; Godovikova, T. I.; Korsunskii, B. L. *Chem. Heterocyclic Comp*. **2003**, *39*, 624.
6. *SMART and SAINT*; Bruker Analytical X-ray Division: Madison, WI, 2002.
7. Sheldrick, G. M. *SHELXTL-PLUS Software Package*; Bruker Analytical X-ray Division, Madison, WI, 2002.
8. Tietze, L. F.; Bothe, U.; Griesbach, U.; Nakaichi, M.; Hasegawa, T.; Nakamura, H.; Yamamoto, Y. *Chem. Bio. Chem*. **2001**, *2*, 326.