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Synthesis and characterization of polar functional group substituted mono- and bis-(*o*-carboranyl)-1,3,5-triazine derivatives

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Abstract—Synthesis, structural characterization, and biological activity studies of *o*-carborane-substituted 1,3,5-triazines (**9**–**12**) containing polar functional groups such as methoxyethyl and *t*-butoxycarbonylmethyl amine units are described. De-methylation of di(methoxyethyl)amine functionalized triazines **9** and **10** resulted in the production of di(hydroxyethyl)amine derivatives **13** and **14**. NMR (^{1}H and ^{13}C) and X-ray crystallographic studies confirmed the structures derived from the sequential *o*-carborane substitution on the 1,3,5-triazine core. Preliminary in vitro studies revealed that compounds **9**, **10**, **13**, and **14**, despite their low cytotoxicity, accumulated at high levels in B-16 melanoma cells.

1,3,5-Triazines are a class of nitrogen-containing heterocyclic compounds with remarkable chemical stability.[1] The stability of these compounds along with their anti-tumor activities has led to their utilization in several specialized biomedical applications.[2] As a surrogate for 1,3,5-triazine, 2,4,6-tris(*N*-methyl-*N*-hydroxymethyl-amino)-1,3,5-triazine (known as trimelamol) was proposed as a potent anti-tumor agent.[3] The 1,3,5-triazine ring has three distinct nucleophilic centers,[4] making it possible to attach various functional groups to the ring by simple nucleophilic substitution reactions at each of the cyanyl chloride (–N=C–Cl) units.[5] It has been demonstrated that *o*-carboranyl anions can function as nucleophiles[6] to facilitate substitution on the carbon atoms of 1,3,5-triazine. Given this behavior, and our previous success[7] in sequentially incorporating *o*-carboranyl units to 1,3,5-triazine, in the present work we sought to utilize the triazine core as a template for the production of potential boron neutron capture therapy (BNCT) agents. For a compound to have potential as

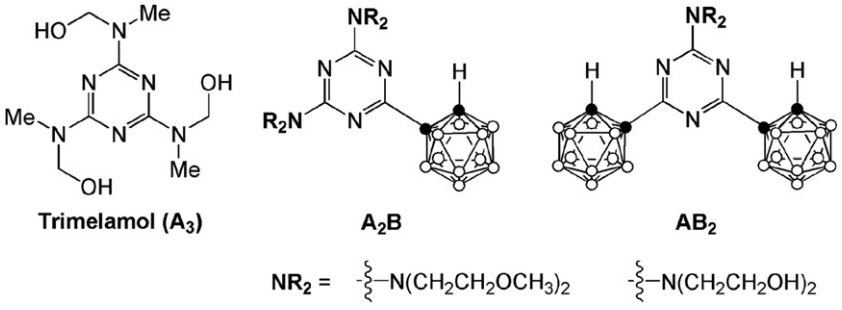

Figure 1. A_2B and AB_2 triazine systems.

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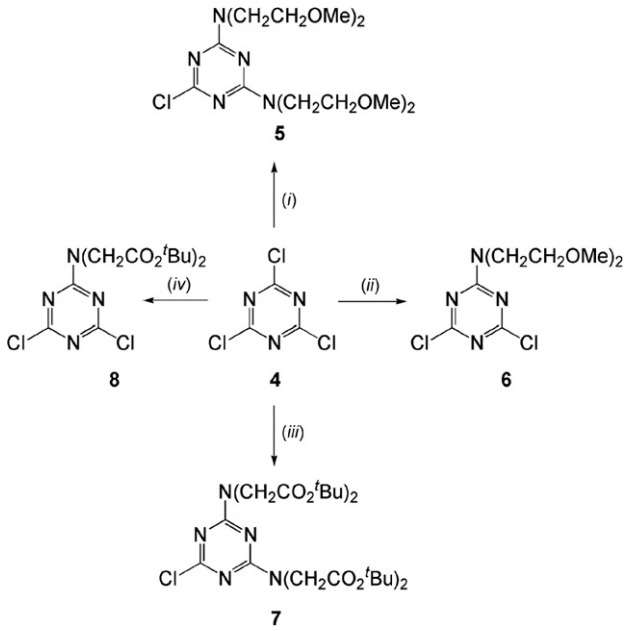
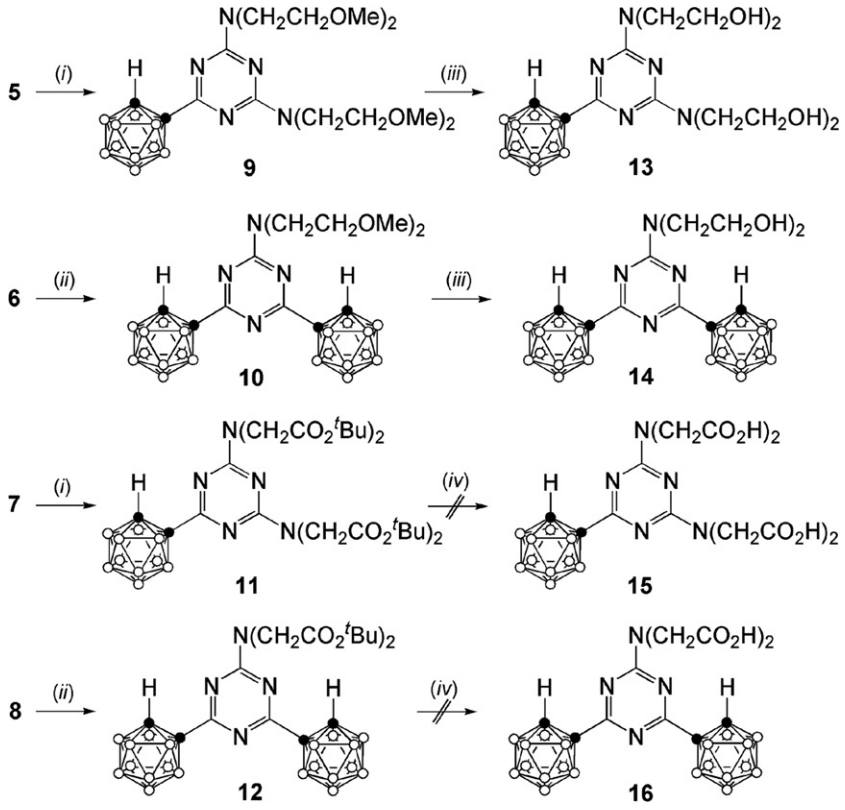


Scheme 1. Reagents and conditions: (i) $HN(CH_2CH_2OCH_3)$ (2 equiv), $(i\text{-Pr})_2EtN$ (2 equiv), THF, rt; (ii) $HN(CH_2CH_2OCH_3)$, $(i\text{-Pr})_2EtN$, THF, −10 °C; (iii) $HN[CH_2CO_2C(CH_3)_2]$ (2 equiv), $(i\text{-Pr})_2EtN$ (2 equiv), THF, rt; (iv) $HN[CH_2CO_2C(CH_3)_2]$, $(i\text{-Pr})_2EtN$, THF, −10 °C.

a BNCT agent, it should be water-soluble, have low cytotoxicity, and take up boron in cancer cells.[8] Due to the lipophilic character of the *o*-carboranyl unit,[9] the introduction of a second functional group into the *o*-carboranyl triazine that endows the molecule with water solubility is highly desirable. The fact that trimelamol, which contains three hydroxyl methyl moieties, is a water-soluble bioactive agent[3] suggests that introducing one or more hydroxyalkyl units to the *o*-carboranyl triazine may enhance its solubility in aqueous solution. As shown in Figure 1, conversion of the second functional group of *o*-carboranyl triazine to a hydroxyethyl group yielded a molecule 10–100 times more soluble in water than previously reported A_2B-type molecules without a polar functional group (7.24×10^{-6} (mol/mL) (av.)),[10] where A and B represent the aminoalkyl- and *o*-carboranyl substituents of the triazine, respectively.

To incorporate polar groups into the triazine system, we first attempted to prepare hydroxyethyl- and hydroxycarbonylmethyl amine surrogates. Thus, a series of mono- and bis-substituted precursors (**5**–**8**) containing di(methoxyethyl)- and di(*t*-butoxycarbonylmethyl)-amine functional groups was prepared by the reaction of compound **4** with di(methoxyethyl)- and di(*t*-butoxycarbonylmethyl)amine, respectively, in 1:1 and 1:2 stoichiometry (Scheme 1).[11]

When lithiated *o*-carborane was reacted with precursors **5**–**8** in 1:1 or 1:2 stoichiometry, the corresponding mono- and di-substituted *o*-carboranyl triazines (**9**–**12**) were formed in 12–80% yield (Scheme 2).[12] Finally, the desired free alcohol species **13** and **14** were prepared in

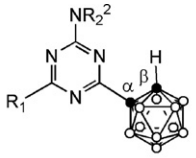


Scheme 2. Reagents and conditions: (i) Lithio-*o*-carborane (1 equiv), THF, −78 °C to rt; (ii) lithio-*o*-carborane (2 equiv), THF, −78 °C to rt; (iii) BBr_3, CH_2Cl_2; (iv) CF_3CO_2H.

57–71% yield by reacting **9** and **10** with BBr_3, respectively.[13] On the other hand, the free acid forms of **15** and **16** were not obtained when we attempted the de-alkylation of **11** and **12** under trifluoroacetic acid conditions; rather, it appeared that **11** and **12** were easily decomposed under acidic conditions.[13]

Selected physical and spectroscopic properties of *o*-carboranyl-1,3,5-triazine derivatives **9**–**14** are listed in Table 1. The presence of the *o*-carboranyl ring was confirmed by the characteristic absorption bands at around 2563–2606 cm^{-1} assignable to B–H bonds in the infrared spectra. In the ^1H NMR spectra of **9**–**14**, signals diagnostic for methylene protons of NCH_2 were observed at around δ 3.56–4.25. Key signals detected in the ^{13}C NMR spectra of **9**–**14** include resonances at around δ 57.2–61.5 (*C*-β), 59.0–69.9 (NCH_2), 72.4–75.7 (*C*-α), and 163.4–175.6 (triazine ring). Sequential

Table 1. Summary of selected physical and spectral properties of the *o*-carboranyl-1,3,5-triazine derivatives **9**–**14**



R_1 = Carboranyl, $N(CH_2CH_2OCH_3)$, $N(CH_2CH_2OH)_2$
R_2 = $N(CH_2CH_2OCH_3)$, $N(CH_2CH_2OH)_2$

No.	Compound	Mp[a] (°C)	Yield[b] (%)	IR (B–H)	NMR ($^1H/^{13}C$)				
					$C(NCH_2)$	$C(OCH_2)$	C(triazine)	C(α)	C(β)
1	**9**	97–98	18	2584	3.57 (m)	3.84 (m)			4.5 (s)
					49.0	69.9	163.4, 167.5	72.7	59.0
2	**10**	120–122	80	2563	3.56 (t)	3.83 (t)			4.42 (s)
					49.0	69.9	163.5, 167.5	72.8	59.0
3	**11**	104–106	12	2606	4.20 (d)				4.43 (s)
					50.5 (d)		164.9, 166.5	73.9	56.2
4	**12**	102–104	72	2582	4.24 (s)				4.36 (s)
					51.2		164.4, 167.2	72.4	56.2
5	**13**	106–107	54	2600	3.81 (t)	3.87 (t)			5.28 (s)
					51.6 (d)	59.0	164.0, 167.3	73.5	57.6
6	**14**	108–110	71	2600	3.81 (t)	3.87 (t)			5.28 (s)
					51.6 (d)	59.0	164.0, 167.3	73.5	57.6

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

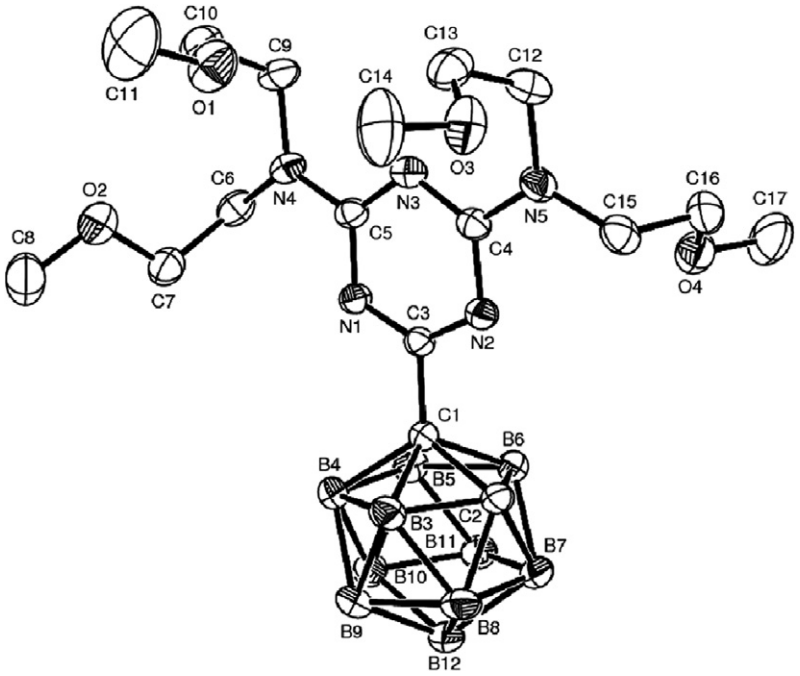


Figure 2. Molecular structure of compound **9**. The thermal ellipsoids are drawn at the 30% probability level.

mono- and bis-substitutions of *o*-carboranyl cages to the triazinyl center were further authenticated through X-ray structural studies of **9** and **10**, respectively (Figs. 2 and 3). The crystal structures determined on the basis of X-ray diffraction data corresponded well with the conformations derived from the NMR spectra.

Taking into consideration the three essential requirements for BNCT precursors—good water solubility, low cytotoxicity, and high boron uptake—**9**, **10**, **13**, and **14** appear to be good candidate molecules (see Table 2).[8] It has been noted that trimelamol shows high cytotoxicity in addition to its high anti-tumor activity. However, four structurally related compounds exhibit low cytotoxicity,[14] with IC_{50} values (the half maximal inhibitory concentration) in the range of 4.49×10^{-5}–6.54×10^{-5} M. Among the series, A_2B systems were more soluble in water than AB_2 systems. Furthermore, as the amino functional group was converted to a more polar substituent with a hydroxyethyl group, the water solubility increased to 5.18×10^{-4} (mol/mL) for **13**, which is about two orders of magnitude higher than the solubilities we observed for A_2B-type molecules with alkylamino functional groups in our previous work.[10] All four compounds prepared in the present work (**9**, **10**, **13**, and **14**) were found to accumulate markedly in B-16 melanoma cells when compared to BPA (*p*-boronophenylalanine). We found no direct correlation between water solubility and boron uptake for this series. We attribute the small variation in boron uptake among the four compounds to their similar lipophilic characters, even when a polar functional group was introduced in the A_2B system such as in **13**.

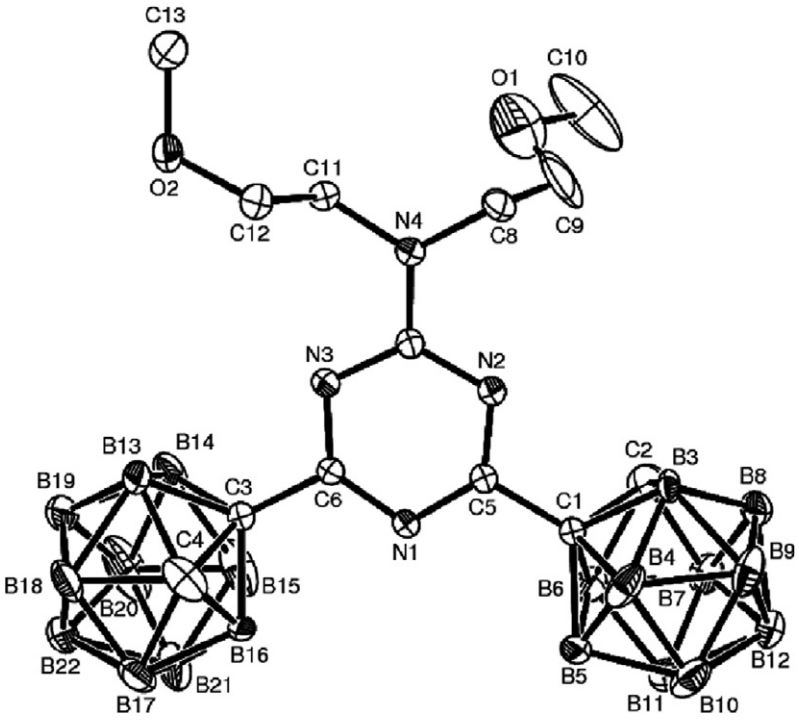


Figure 3. Molecular structure of compound **10**. The thermal ellipsoids are drawn at the 30% probability level.

Table 2. Cytotoxicity (IC_{50}) of the test compounds toward B-16 cells and boron uptake

Compound		B-16[a] (IC_{50}) M	Boron uptake[b] (μg B/10^6 cells)		Water solubility (mol/mL)
1	**9**	4.49×10^{-5} (± 0.30)	2.55	± 0.84	3.27×10^{-6}
2	**10**	6.54×10^{-5} (± 0.07)	2.01	± 0.37	1.51×10^{-6}
3	**13**	4.71×10^{-5} (± 0.33)	1.81	± 0.81	5.18×10^{-4}
4	**14**	4.75×10^{-5} (± 0.11)	2.16	± 2.56	1.03×10^{-5}
	BPA	4.49×10^{-5} (± 0.30)	0.083	± 0.012	

[a] B-16: B-16 melanoma cells.

[b] Boron uptake by B-16 cells was determined using the ICP-AES method (see Ref. 15). Briefly, cells were cultured in Falcon dishes (90 mm∅) until they grew to fill the dishes (~3.0×10^6 cells/dish). Cells were then incubated for 3 h with Eagle–MEM medium containing one of the test compounds (boron concentration: 10.8 ppm). After 3 h, the cells were washed three times with PBS(—) and processed for the determination of the boron concentration by ICP-AES. Each experiment was carried out in triplicate.

Electronic supplementary information (ESI) available: experimental details and spectral data for **9**, **10**, **11**, **12**, **13** and **14**, X-ray crystallographic data for **9** and **10** (CCDC No. 655714 and 655715).

Acknowledgments

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10. Unpublished results.
11. *6-Chloro-2,4-bis[di(2-methoxyethyl)amino]-1,3,5-triazine (5)*: To a stirred solution of cyanuric chloride **4** (1.84 g, 10 mmol) and *N,N*-diisopropylethylamine (2.58 g, 20 mmol) in 30 mL of THF at −10 °C was added di(2-methoxyethyl)amine (2.66 g, 20 mmol) via a syringe. The reaction temperature was maintained at −10 °C for 1 h, after which the reaction mixture was warmed slowly to room temperature. The mixture was then stirred for an additional 12 h, after which it was quenched with distilled H_2O (50 mL). The crude product was then 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 give 3.62 g (96%) of **5**. Mp 94–95 °C. ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 6H), 3.58 (t, $J = 5.5$ Hz, 4H), 3.86 (t, $J = 5.5$ Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 48.6, 59.0, 70.1, 164.8, 169.9. *Compound* **6**: Yield. 91%. Mp 54–56 °C. ^{1}H NMR ($CDCl_3$) δ 3.32 (s, 12H), 3.57 (t, $J = 5.5$ Hz, 8H), 3.85 (t, $J = 5.5$ Hz, 8H). ^{13}C NMR ($CDCl_3$) δ 48.6, 58.9, 70.1, 164.8, 169.9. *Compound* **7**: Yield. 96%. Mp 158–160 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 36H), 4.15 (d, $J = 45.8$ Hz, 8H). ^{13}C NMR ($CDCl_3$) δ 28.11–28.15, 50.0–50.2, 81.9–82.0, 165.4, 168.3–168.5, 169.3. *Compound* **8**: Yield. 94%. Mp 65–66 °C. ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 18H), 4.23 (d, $J = 47.2$ Hz, 4H). ^{13}C NMR ($CDCl_3$) δ 28.1, 50.0–50.2, 81.9, 165.4, 168.3–168.5, 169.3.
12. *6-(o-Carboran-1-yl)-2,4-bis[di(2-methoxyethyl)amino]-1,3,5-triazine (9)*: To a stirred solution of *o*-carborane (1.44 g, 10 mmol) in 30 mL of THF at −78 °C was added 2.5 M *n*-BuLi (4.0 mL, 10 mmol) via a syringe. A solution of compound **5** (3.78 g, 10 mmol) in THF was slowly added to the reaction flask at −78 °C, and the reaction temperature was maintained at −78 °C for 1 h. The reaction mixture was then warmed slowly to room temperature, stirred for an additional 12 h, and quenched with distilled H_2O (30 mL). The crude product was then 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. Product **9** was isolated by flash column chromatography (ethylacetate/hexane 1:8) in 18% yield (0.86 g). Mp 97–98 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2584. ^{1}H NMR ($CDCl_3$) δ 3.33 (s, 6H), 3.34 (s, 6H), 3.57 (m, $J = 5.1$ Hz, 8H), 3.84 (m, $J = 5.1$ Hz, 8H), 4.50 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 49.0, 56.1, 59.0, 69.9, 72.7, 163.4, 167.5. Compound **10**: Yield. 80%. Mp 120–122 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2563. ^{1}H NMR ($CDCl_3$) δ 3.34 (s, 6H), 3.56 (t, $J = 5.5$ Hz, 4H), 3.83 (t, $J = 5.5$ Hz, 4H), 4.41 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 49.0, 56.1, 59.0, 69.9, 72.7, 163.4, 167.5. Compound **11**: Yield. 12%. Mp 104–106 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2606, ν (C–H) 1747. ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 18H), 1.45 (s, 18H), 4.15 (d, $J = 45.8$ Hz, 8H), 4.43 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.1, 50.2–50.5, 56.2, 73.9, 82.1, 164.9, 166.5, 168.4–168.5. *Compound* **12**: Yield. 72%. Mp 102–104 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2582, ν (C–H) 1743. ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 18H), 4.24 (s, 4H), 4.36 (s, 1H). ^{13}C NMR ($CDCl_3$) δ 28.1, 51.2, 56.2, 72.4, 83.3, 164.4, 167.2, 167.9.
13. *6-(o-Carboran-1-yl)-2,4-bis[di(2-hydroxyethyl)amino]-1,3,5-triazine (13)*: To a stirred solution of compound **9** (2.43 g, 5 mmol) in 20 mL of THF at −10 °C was added BBr_3 (5.01 g, 20 mmol) via a syringe. The reaction temperature was maintained at −10 °C for 30 min, after which the reaction mixture was warmed slowly to room temperature. After stirring for an additional 2 h, the reaction was quenched with distilled H_2O (50 mL). The crude product was extracted with diethyl ether (50 mL × 2). The organic layer was washed with H_2O and then dried in vacuo. Product **13** was isolated by flash column chromatography (ethylacetate/hexane 1:2) in 54% yield (1.16 g). Mp 106–107 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2600. ^{1}H NMR (acetone-d_6) δ 3.81 (t, $J = 5.1$ Hz, 8H), 3.87 (t, $J = 5.7$ Hz, 8H), 5.28 (s, 1H). ^{13}C NMR (acetone-d_6) δ 51.2–51.6, 57.6, 59.0, 73.5, 164.0, 167.3. *Compound* **14**: Yield. 71%. Mp 108–109 °C. IR (KBr pellet, cm^{-1}) ν (B–H) 2600. ^{1}H NMR (acetone-d_6) δ 3.81 (t, $J = 5.05$ Hz, 4H), 3.87 (t, $J = 5.7$ Hz, 4H), 5.28 (s, 1H). ^{13}C NMR (acetone-d_6) δ 51.2–51.6, 57.6, 59.0, 73.5, 164.0, 167.3.

Compounds **15** and **16**: A trifluoroacetic acid (8 mL) solution containing compound **11** (1.41 g, 2 mmol) or **12** (1.22 g, 2 mmol) was stirred for 24 h at room temperature and then dried in vacuo. However, we did not obtain the de-alkylated compounds.

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