DOI: 10.1002/ejoc.201000132

Synthesis of Hyperbranched β-Galceramide-Containing Dendritic Polymers that Bind HIV-1 rgp120

José Antonio Morales-Serna,[a] Omar Boutureira,[a] Angels Serra,[a] M. Isabel Matheu,[a] Yolanda Díaz,[a] and Sergio Castillón*[a]

Keywords: Polymers / Hyperbranched polymers / Glycolipids / Glycoconjugates / Dendrimers / Galceramide / HIV

We report the design, synthesis, and characterization of a series of water-soluble hyperbranched β-galceramide-containing dendritic. Polymers showing useful binding ability to HIV-1 rgp120 as demonstrated with surface plasmon resonance.

Introduction

Hyperbranched polymers represent an important part of the family of dendritic and multibranched polymers, whose properties are strongly determined by the nature of their terminal groups.[1] For example, solubility, which mainly depends on the end group structure, may be regulated by the partial or total chemical modification of these terminal groups.[2] Hyperbranched polymers can be easily synthesized via one-step reactions and, therefore represent economically promising products for both small- and large-scale industrial processes. A wide variety of applications which originally only seemed conceivable for dendrimers have been investigated for statistically branched and hyperbranched polymers.[3] Among these new potential applications, their use as drug-delivery systems, macromolecular carriers, and biomimetic materials seem to be one of the most challenging goals.[4] In particular, the highly branched structures allow for the multivalent presentation of ligands and therefore will play a key role in host-guest recognition processes. These engineered nanomaterials could be envisioned as macromolecular agents able to interact with biological systems and potentially have uses in medicine and biotechnology. This idea has been previously exploited in a series of studies with Boltorn® H30 (**1**), a hyperbranched dendritic polymer (Figure 1) functionalized with D-mannose. This glycopolymer exhibits interesting binding properties with *Lens culinaris* lectin[5] and has been proven as an effective inhibitor of DC–SIGN-mediated infection in an Ebola-pseudo-typed viral model.[6]

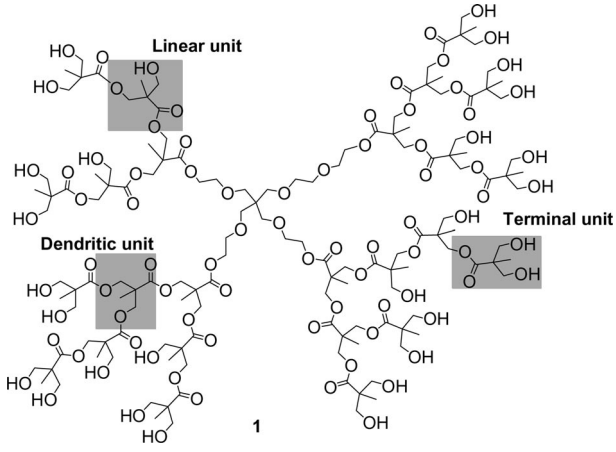


Figure 1. Idealized chemical structure of commercial hyperbranched polymer Boltorn® H30 (**1**).

However, these studies have always been performed with discrete carbohydrates linked through a spacer to the dendrimer core. No complex naturally occurring structures have been utilized despite the fact that other functionalities present in such natural products (e.g. lipophilic chains) play an important role and sometimes are ultimately responsible for their biological behavior (e.g. insertion of lipophilic units into lipid rafts).[7] To the best of our knowledge, a general strategy for the synthesis of hyperbranched dendritic polymers functionalized with naturally occurring β-galceramide (β-galcer) has not been reported. Interestingly the intrinsic microheterogeneity of these hyperbranched dendritic polymers could potentially mimic microdomain formation in lipid membranes which is typically associated with important cellular events such as signaling, vesicle fusion, and pathogen invasion amongst others.[8] Studies described in the literature illustrate that glycosphingolipids like β-galcer and GM3, play a important role during HIV-infection.[9] To address this challenge and study the interac-

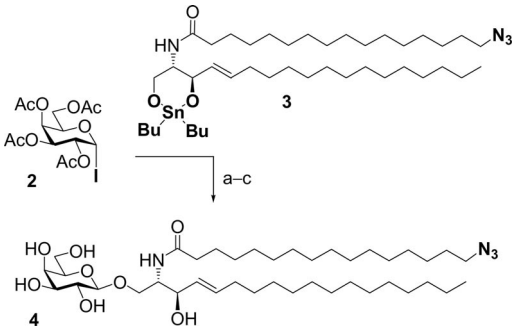
[a] Departament de Química Analítica i Química Orgànica, Universitat Rovira i Virgili,
c/Marcel.lí Domingo s/n, 43007 Tarragona, Spain
Fax: +34-977-558446
E-mail: sergio.castillon@urv.cat

Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/ejoc.201000132.

tion of HIV-1 rgp120 with β-galcer-containing dendrimers with the aim of developing potential carbohydrate-based inhibitors, multivalent scaffolds bearing glycosphingolipids have been prepared. These systems consist of dendritic hyperbranched structures based on Boltorn® H30 **1** to which β-galcer-N_3 units **4** are attached using Cu^I-catalyzed [3+2] alkyne-azide cycloaddition (CuAAC). This system has previously been used as key step for the efficient synthesis of dendrimers[10] and the functionalization of hyperbranched aliphatic polyols.[11] This is mainly due to the enhanced complexity of hyperbranched materials raising the demand for robust and versatile synthetic methods. The following characteristics make CuAAC reaction a powerful tool for the modification of hyperbranched polymers: a) reaction robust and quantitative; b) able to proceed in various solvents and highly tolerant to other functional groups; c) the reaction proceeds at various types of interfaces; d) virtually no formation of by-products; and e) easy to handle and purify.[1b]

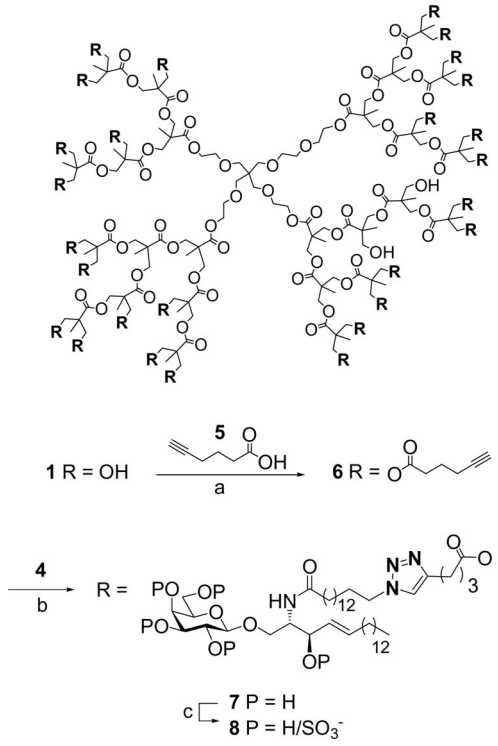
Results and Discussion

Our initial efforts were directed toward the synthesis of compound **4** from tetra-*O*-acetyl-α-iodogalactose **2** and stannyl ceramide **3** (Scheme 1). Thus following the methodology reported in our group,[12,13] a mixture of tetra-*O*-acetyl-α-iodogalactose **2** and stannyl ceramide **3** was treated with TBAI in toluene and heated at 80 °C for 18 h. This initially afforded the corresponding orthoester which was further isomerized with $BF_3{\cdot}OEt_2$ to the final acetylated β-galcer derivative. Finally, Zemplén deacetylation, afforded glycosphingolipid **4** in high yield (88% over three steps) and complete regio (OH-1) and β-selectivity (Scheme 1). Moreover this simple transformation provides a solution to the long-standing problem of direct glycosylation of ceramides and sugars, reduces the overall number of steps, and gives rapid access to biologically important β-glycolipids and their derivatives.



Scheme 1. Reagents and conditions: a) TBAI, toluene, 80 °C, 18 h; b) $BF_3{\cdot}Et_2O$, CH_2Cl_2, 0 °C, 3 h; c) NaOMe, MeOH, room temperature, 12 h (88% over three steps); TBAI = tetra-*n*-butylammonium iodide.

As previously noted, click chemistry[14] and in particular CuI-catalyzed 1,2,3-triazole formation from azides and terminal acetylenes has proven to be a powerful tool in most aspects of drug discovery[15] as well as in the preparation of water-soluble glycoconjugates.[16] Therefore, we used this strategy to attach our target molecules to the dendritic core. This was achieved by mixing Boltorn® H30 (**1**) and 5-hexynoic acid (**5**) in the presence of EDC and Et_3N (Scheme 2). Thus, functionalized hyperbranched **6** was obtained, as demonstrated by ^1H and ^{13}C NMR and MALDI–TOF MS analysis (see Supporting Information).



Scheme 2. Reagents and conditions: a) EDC, Et_3N, CH_2Cl_2, room temperature, 5 d; EDC = *N*-(3-dimethylaminopropyl)-*N*′-ethylcarbodiimide. b) $CuSO_4$, *t*BuOH/H_2O, sodium ascorbate, room temperature, 72 h; c) $SO_3{\cdot}Me_3N$, DMF, 60 °C, 24 h.

Dendritic, linear, and terminal units can be distinguished by ^{13}C NMR in Boltorn® H30 (**1**) by identifying the quaternary carbon atoms between 50.25–46.25 ppm.[17] After partial modification of OH groups with alkyne **5**, new pseudo-dendritic and pseudo-linear units were generated. This evolution is visible with a decrease in the intensity of the terminal signals at δ = 50.25 ppm, as well as the appearance of news peaks in the linear and dendritic regions between 48.5 and 46.2 ppm (Figure 2). A quantitative analysis of these signals allowed the estimation of the total amount of hexynoic acid that was initially added to Boltorn® H30 from the integration of the quaternary carbon atoms[17] (62%).

Next a mixture of β-galcer-N_3 (**4**) and the hyperbranched polymer **6** was treated for 72 h with sodium ascorbate and $CuSO_4$ in *t*BuOH/H_2O[18] (Scheme 2). After dialysis the corresponding glycodendritic β-galcer-containing polymer **7** was obtained. This process allowed anchoring of unprotected β-galcer units to a dendritic polymer leading to a new class of glycoconjugates: "glycosphingodendrimers".

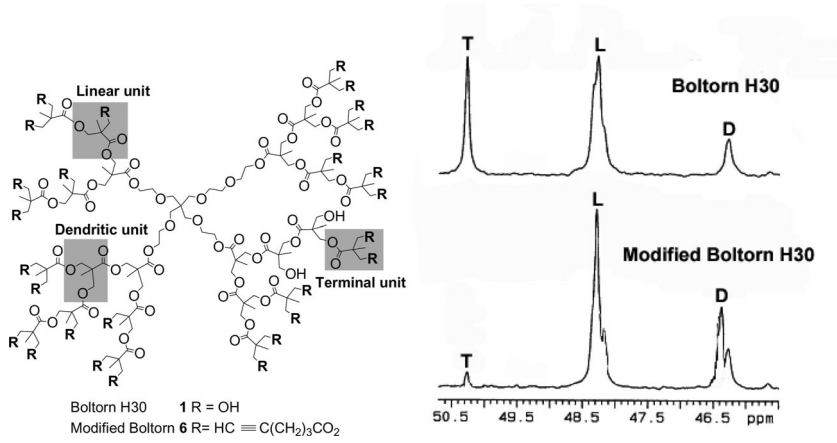


Figure 2. Cq area in the ^{13}C NMR spectra of dendritic polymers **1** and **6**; terminal (T), linear (L), and dendritic (D).

The analysis of the ^{1}H NMR spectra of glycodendritic polymer of β-galcer **7** revealed the presence of the triazole protons at δ = 7.79 ppm and complete disappearance of the acetylenic signal at δ = 1.99 ppm. In the ^{13}C NMR spectra it is possible to observe signals of the 4- and 5-position of 1,2,3-triazol which appears at δ = 145 ppm and 126.1 ppm. Signals indicative of the double bond can be observed at δ = 134.6 ppm and 130.2 ppm, whereas the anomeric carbon from D-galactose appears at δ = 105 ppm (see Supporting Information).

Compound **7** was subjected to sulfation by treatment with an excess of $SO_3{\cdot}Me_3N$. After dialysis sulfated glycodendritic compound **8** was obtained (Scheme 2). ^{13}C NMR analysis revealed a new peak at δ = 81.6 ppm, which can be attributed to the sulfated carbon of D-galactose. MALDI–TOF MS analysis of the hyperbranched polymers revealed that 62.84% of the **1** was derivatized with **5**. In the case of **7**, on average 19.92 β-galcer residues were incorporated (95.47%) onto the dendritic polymer **6**. Analogously, on average 20.04 sulfate groups were added to the glycodendritic polymer **7** to afford **8** (Table 1).

Table 1. MALDI–TOF MS analysis of **6**, **7**, and **8**.

	Theoretical molecular wt.	Observed molecular wt.	Average no. of residues[a]	Theoretical incorporation
6	6392	5275.9	20.11	62.84
7	200064	20033.9	19.92	95.47
8	21644	21617.1	20.04	100.60

[a] See Suporting Information for further details.

The HIV-1 entry and infection involves a step-wise process that includes a number of host cell proteins and lipids. This process is coordinated by the HIV-1 envelope glycoprotein complex (trimer of gp120 surface glycoproteins) each noncovalently attached to three gp41 membrane glycoprotein subunits, that interact with receptors CD4, CXCR4, and CCR5 on the host cell surface.[19] Reliable strategies have been developed to design glycoconjugate based molecules that inhibit HIV-1 fusion.[20] The synthesis of multivalent neoglycoconjugates is currently promoted by the extensive findings of multiple ligand-receptor interactions that occur in nature and by the phenomenon generally referred to as the glycoside cluster effect.[21]

In this context to demonstrate the binding ability of hyperbranched β-galceramide-containing dendritic polymers **7** and **8** toward HIV-1 rgp120 IIIB, a kinetic analysis was performed using surface plasmon resonance (SPR). The rate and affinity constants for hyperbranched-rgp120 IIIB interactions are shown in Table 2. This data suggests that rgp120 IIIB exhibits similar binding to hyperbranched β-galceramide-containing dendritic polymers **7** and **8**. Additionally, this results are close to those obtained with the glycodendrimers galcer (GC-32mer with an average of 21 sugars) (entry 3, Table 2), and lower than sulfated galcer (SGal-32mer with an average of 25 sugars).[22–24]

Table 2. Rate and equilibrium constants for the interaction of rgp120 IIIB with hyperbranched glycodendritic β-galceramide-containing polymers **7** and **8**.

Compound	k_a	k_d	K_D(M)
7	6.4×10^{5}	5.9×10^{-3}	9.14×10^{-8}
8	3.6×10^{4}	4.6×10^{-3}	1.28×10^{-8}
GC-32mer with 21 sugars[a]	3.7×10^{5}	1.03×10^{-3}	5.94×10^{-9}
SGal-32mer with 25 sugars[a]	6.27×10^{5}	1.18×10^{-4}	1.89×10^{-10}

[a] Ref.[22]

On the other hand, the ability of hyperbranched glycodendritic polymers to inhibit HIV-1 BaL (R5-tropic) infection of U373-MAGI-CCR5 cells and their effects on cell viability was tested (see Supporting Information). The observed EC_{50} value of 80 μM for compound **8** is consistent with the values recently reported in similar structures.[21] In contrast, low inhibition was observed with glycodendritic polymer **7**. The fact that **7** and **8** have different behavior in the inhibition can be justified by the presence of sulfate groups in **8**.[19a]

Conclusions

In summary, we report the synthesis of water-soluble hyperbranched β-galceramide-containing dendritic polymers which consists of Boltorn® H30 core to which naturally occurring β-galceramide units have been covalently attached. These promising glycodendritic polymers have been used as models to mimic multivalent glycosphingolipid display on cell surfaces, demonstrating an excellent cluster effect. This is in agreement with the current hypothesis that the assembly of the HIV-1 entry complex requires, in addition to CD4 and a co-receptor, specific glycolipid rafts which are present in restricted areas of the plasma membrane. This approach opens new routes to the development of novel classes of HIV-1 binding antagonists employing hyperbranched dendritic polymers as new biomaterials.

Supporting Information (see also the footnote on the first page of this article): General experimental methods, experimental procedures, compound characterization data, and NMR spectra for all new compounds.

Acknowledgments

Financial support of this work by the Spanish Ministerio de Educación y Ciencia (MEC) (DGSIC CTQ2008-01569/BQU) is acknowledged. Support from the Generalitat de Catalunya and Fons Social Europeu (to J. A. M. S. and O. B.) is gratefully acknowledged. We are also grateful to the Servei de Recursos Científics (URV) for its technical assistance.

[1] a) B. I. Voit, A. Lederer, *Chem. Rev.* **2009**, *109*, 5924–5973; b) A. Carlmark, C. Hawker, A. Hult, M. Malkoch, *Chem. Soc. Rev.* **2009**, *38*, 352–362; c) B. Voit, *J. Polym. Sci., Part A: Polym. Chem.* **2005**, *43*, 2679–2699; d) C. Gao, D. Yan, *Prog. Polym. Sci.* **2004**, *29*, 183–275.

[2] a) R. Scherrenberg, B. Coussens, P. van Vliet, G. Edouard, J. Brackman, E. de Brabander, *Macromolecules* **1998**, *31*, 456–461; b) B. I. Voit, *Acta Polym.* **1995**, *46*, 87–99.

[3] a) R. Haag, *Angew. Chem.* **2004**, *116*, 280–284; *Angew. Chem. Int. Ed.* **2004**, *43*, 278–282; b) R. Haag, *Chem. Eur. J.* **2001**, *7*, 327–335.

[4] a) C. Gao, Y. M. Xu, D. Y. Yan, W. Chen, *Biomacromolecules* **2003**, *4*, 704–712; b) P. Kolhe, E. Misra, R. M. Kannan, S. Kannan, M. Lieh-Lai, *Int. J. Pharm.* **2003**, *259*, 143–160; c) T. Ooya, J. Lee, K. Park, *J. Control. Release* **2003**, *93*, 121–127; d) H. Frey, R. Haag, *Rev. Mol. Biotechnol.* **2002**, *90*, 257–267; e) R. M. Crooks, *ChemPhysChem* **2001**, *2*, 644–654.

[5] E. Arce, P. M. Nieto, V. Díaz, R. G. Castro, A. Bernad, J. Rojo, *Bioconjugate Chem.* **2003**, *14*, 817–823.

[6] a) J. Rojo, R. Delgado, *Antimicrob. Agents Chemother.* **2004**, *54*, 579–581; b) F. Lasala, E. Arce, J. R. Otero, J. Rojo, R. Delgado, *Antimicrob. Agents Chemother.* **2003**, *47*, 3970–3972.

[7] a) D. Wilflingseder, H. Stoiber, *Front. Biosci.* **2007**, *12*, 2124–2135; b) J. Fantini, N. Garmy, R. Mahfoud, N. Yahi, *Expert Rev. Mol. Med.* **2002**, *4*, 1–22.

[8] K. Jacobson, O. G. Mouritsen, R. G. W. Anderson, *Nat. Cell Biol.* **2007**, *9*, 7–14.

[9] a) J. Fantini, D. G. Cook, N. Nathanson, S. L. Spitalnik, F. Gonzalez-Scarano, *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 2700–2704; b) D. Hammache, G. Piéroni, N. Yahii, O. Delézay, N. Kochi, H. Lafont, C. Tamaleti, J. Fantini, *J. Biol. Chem.* **1998**, *273*, 7967–7971.

[10] P. Wu, M. Malkoch, J. N. Hunt, R. Vestberg, E. Kaltgrad, M. G. Finn, V. V. Folkin, K. B. Sharpless, C. J. Hawker, *Chem. Commun.* **2005**, 5775–5775.

[11] A. R. Katritzky, Y. Song, R. Sakhuja, R. Gyanda, N. K. Meher, L. Wang, R. S. Duran, D. A. Ciaramitaro, C. D. Bedford, *J. Poym. Sci., Part A: Polym. Chem.* **2009**, *47*, 3748–3756.

[12] Sphingosine Synthesis: a) J. Llaveria, Y. Díaz, M. I. Matheu, S. Castillón, *Org. Lett.* **2009**, *11*, 205–208; b) J. A. Morales-Serna, M. I. Matheu, Y. Díaz, S. Castillón, *Synthesis* **2009**, 710–712; c) J. A. Morales-Serna, J. Llaveria, M. Matheu, Y. Díaz, S. Castillón, *Org. Biomol. Chem.* **2009**, *7*, 4502–4504.

[13] Glycosyl ceramide synthesis: a) J. A. Morales-Serna, M. I. Matheu, Y. Díaz, S. Castillón, *Eur. J. Org. Chem.* **2009**, 3849–3852; b) J. A. Morales-Serna, Y. Díaz, M. I. Matheu, S. Castillón, *Org. Biomol. Chem.* **2008**, *20*, 3831–3836; c) O. Boutureira, J. A. Morales-Serna, Y. Díaz, M. I. Matheu, S. Castillón, *Eur. J. Org. Chem.* **2008**, 1851–1854; d) J. A. Morales-Serna, O. Boutureira, Y. Díaz, M. I. Matheu, S. Castillón, *Org. Biomol. Chem.* **2008**, *6*, 443–446.

[14] a) H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[15] H. C. Kolb, K. B. Sharpless, *DDT* **2003**, *8*, 1128–1136.

[16] a) J.-F. Lutz, H. G. Börner, *Prog. Polym. Sci.* **2008**, *31*, 1–39; b) J.-F. Lutz, *Angew. Chem.* **2007**, *120*, 2212–2214; *Angew. Chem. Int. Ed.* **2007**, *46*, 2182–2184; c) V. V. Foking, *ACS Chem. Biol.* **2007**, *2*, 775–778.

[17] E. Žagar, M. Žigon, S. Podzimek, *Polymer* **2006**, *47*, 166–175.

[18] V. V. Rostovtsev, L. G. Green, V. V. Fokin, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 2708–2711; *Angew. Chem. Int. Ed.* **2002**, *41*, 2596–2599.

[19] a) C. C. Huang, M. Thang, M. Y. Zhang, S. Majeed, E. Montabana, R. L. Stanfield, D. S. Dimitrov, B. Krober, J. Sodroski, I. A. Wilson, R. Wyatt, P. D. Kwong, *Science* **2005**, *310*, 1025–1028; b) B. Chen, E. M. Vogan, H. Gong, J. J. Skehel, D. C. Wiley, S. C. Harrison, *Nature* **2005**, *433*, 834–841.

[20] a) F. Baleux, L. Loureiro-Morais, Y. Hersant, P. Clayette, F. Arenzana-Seisdedos, D. Bonnaffé, H. Lortat-Jacob, *Nat. Chem. Biol.* **2009**, *5*, 743–748; b) K. D. McReynolds, J. Gervay-Hague, *Chem. Rev.* **2007**, *107*, 1533–1552; c) R. Villard, D. Hammache, G. Delapierre, F. Fotiadu, G. Buono, J. Fantini, *ChemBioChem* **2002**, *3*, 517–525.

[21] a) J. J. Lundqist, E. J. Toone, *Chem. Rev.* **2002**, *102*, 555–578; b) M. Mammen, S. K. Choi, G. M. Whitesides, *Angew. Chem.* **1998**, *110*, 2908–2953; *Angew. Chem. Int. Ed.* **1998**, *37*, 2754–2794; c) M. S. Quesenberry, R. T. Lee, Y. C. Lee, *Biochemistry* **1997**, *36*, 2724–2732.

[22] R. K. Kensinger, B. C. Yowler, A. J. Benesi, C.-L. Schengrund, *Bioconjugate Chem.* **2004**, *15*, 349–358.

[23] For a study on the interaction of GalCer with gp120 using fluorescence microscopy see: a) J. C. Conboy, K. D. McReynolds, J. Gervay-Hague, S. S. Saavedra, *Angew. Chem. Int. Ed.* **2000**, *39*, 2882–2884; b) J. C. Conboy, K. D. McReynolds, J. Gervay-Hague, S. S. Saavedra, *J. Am. Chem. Soc.* **2002**, *124*, 968–977.

[24] For a study on the interaction of amphiphilic anionic analogues of galactosylceramide with gp120 using surface-pressure measurements see: B. Faraoux-Corlay, J. Greiner, R. Terrerux, D. Cabrol-Bass, A. M. Aubertin, P. Vierling, J. Fantini, *J. Med. Chem.* **2001**, *44*, 2188–2203.

Received: January 31, 2010
Published Online: April 6, 2010

