Organic Process Research & Development **2009**, *13*, 1156–1160

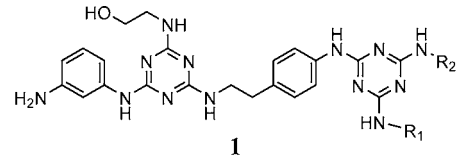
An Efficient Kilogram-Scale Synthesis of *N,N′*-Bis(4,6-disubstituted 1,3,5-triazin-2-yl)-4-aminophenetylamine

Jean-Simon Duceppe, Valérie Perron, Josée Cloutier, Christopher Penney, and Boulos Zacharie*

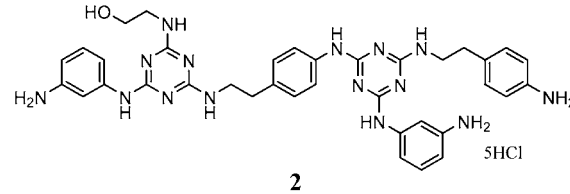
ProMetic BioSciences Inc., 500 boulevard Cartier Ouest, Bureau 150, Laval, Québec, Canada H7V 5B7

Abstract:

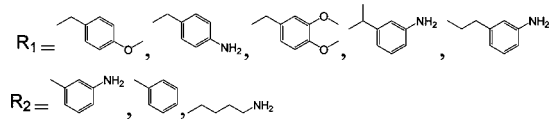
A practical large-scale synthesis was developed for the preparation of *N,N′*-bis(4,6-disubstituted 1,3,5-triazin-2-yl)-4-aminophenethylamine derivatives exemplified by compound 2. This route is a six-step procedure starting from commercially available cyanuric chloride and 3-(*tert*-butoxycarbonylamino) aniline based on the reactivity differences of the three chlorine atoms on the triazine ring. The optimized procedure was scaled up to give the final product 2 in an overall yield of 61% and 99.9% purity. These low-molecular weight compounds mimic the ability of protein A to bind to IgG antibody.



1



2



Introduction

Protein A (MW = 42,000) is found on the surface of the bacteria *Staphylococcus aureus* and binds with high affinity to the tail portion of human and mouse antibodies.[1] These binding properties make protein A commercially important for the purification of monoclonal antibodies. However, purification with protein A affinity absorbents is costly, and these columns can leak protein A and contaminate purified antibodies. Few synthetic compounds have been reported in the literature which can replace protein A and then only when these molecules are attached to solid-phase matrices.[2] Protein A also has therapeutic utility,[3] but its toxicity and cost limit this use.[4] As such, there is a need for a novel, safe, nontoxic small-molecule mimetic of protein A which is economical, stable, and can be administrated as a drug.[5] We have identified a series of low-molecular weight triazine compounds that mimic protein A which are exemplified by the general structure **1**.

Process Research

Large amounts of various high-purity triazines **1** were required for our therapeutic program. Consequently, there was a need to develop a simple, robust general process for the preparation of these compounds. Numerous routes were evaluated for their synthesis on the basis of the reactivity differences of the three leaving groups on the triazine ring. In the case of cyanuric chloride, the substitution of chlorine can be controlled by temperature to run in a stepwise manner. An empirical rule is that monosubstitution of chlorine occurs below or at 0 °C, disubstitution at room temperature, and trisubstitution above 60 °C.[6] Thus, an efficient formation of compound **1** could be considered using the order of substitution of chlorine atoms with different amines. Our efforts resulted in the general synthesis illustrated in Scheme 2 where compound **2** was selected as a representative example of *N,N′*-bis(4,6-disubstituted 1,3,5-triazin-2-yl)-4-aminophenethylamine derivatives. More than 50 analogues of this compound were synthesized using this procedure. A retrosynthetic analysis of protected **2** (compound **7**, Scheme 1) reveals a disconnection of compound **6** to give a monosubstituted dichloro-triazine moiety derived from the displacement of one chlorine atom in cyanuric chloride by monoprotected aminoaniline. The other entity, alcohol **5**, can be seen as derived from substitution of the three chlorines in cyanuric chloride by different nucleophiles in a stepwise manner.

In order to supply interim amounts of **2** for biological testing, the first batches were prepared on a gram scale. This work

* Author for correspondence. E-mail: b.zacharie@prometic.com.

(1) (a) Langone, J. J. *Advances in Immunology*; Dixon, F. J., Kunkel, H. G., Eds.; Academic Press: New York, 1982; Vol. 32, pp 157−252. (b) Nilsson, B.; Abrahmsén, L. *Methods Enzymol.* **1990**, *185*, 144–161. (c) Moks, T.; Abrahmsén, L.; Nilsson, B.; Hellman, U.; Sjoquist, J.; Uhlén, M. *Eur. J. Biochem.* **1986**, *156*, 637–643.

(2) (a) Li, R.; Dowd, V.; Stewart, D. J.; Burton, J.; Lowe, C. R. *Nat. Biotechnol.* **1998**, *16*, 190–195. (b) Fern Teng, S.; Sproule, K.; Hussain, A.; Lowe, C. R. *J. Mol. Recognit.* **1999**, *12*, 67–75. (c) Delano, W. L.; Ultsch, M. H.; de Vos, A. M.; Wells, J. A. *Science* **2000**, *287*, 1279–1283. (d) Ehrlich, G. K.; Bailon, P. *J. Biochem. Biophys. Methods* **2001**, *49*, 443–454. (e) Fassina, G.; Verdoliva, A.; Oderna, M. R.; Ruvo, M.; Cassini, G. *J. Mol. Recognit.* **1996**, *9*, 564–569. (f) Johnson, C. P.; Jensen, I. E.; Prakasam, A.; Vijayendran, R.; Leckband, D. *Bioconjugate Chem.* **2003**, *14*, 974–978.

(3) Sasso, E. H.; Merrill, C.; Furst, D. E. *Ther. Apheresis* **2001**, *5*, 84–91. (b) Brunner, J.; Kern, P. M.; Gaipl, U. S.; Voll, R. E.; Kalden, J. R.; Wiesenhutter, C. W.; Hermann, M. *Mod. Rheumatol.* **2005**, *15*, 9–18.

(4) Silverman, G.; Goodyear, C. S.; Siegel, D. L. *Transfusion* **2005**, *45*, 274–280.

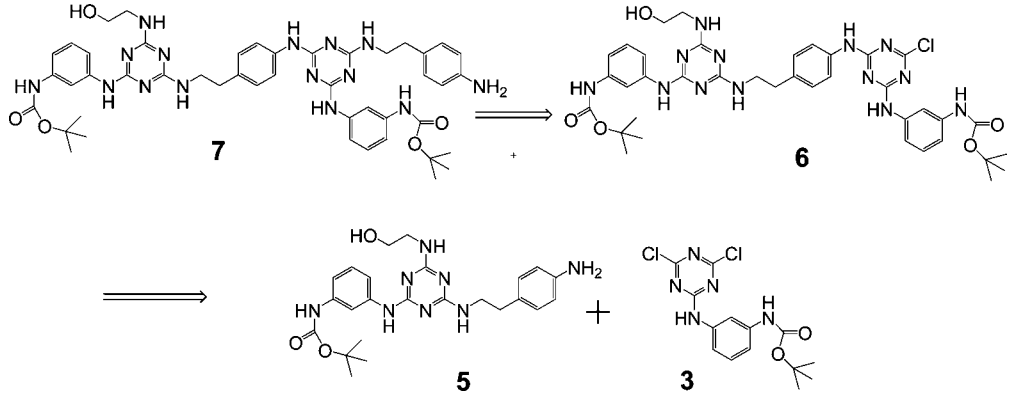
(5) Zacharie, B.; Fortin, D.; Wilb, N.; Bienvenue, J.-F.; Asselin, M.; Grouix, B.; Penney, C. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 242–246.

(6) Blotny, G. *Tetrahedron* **2006**, *62*, 9507–9522.

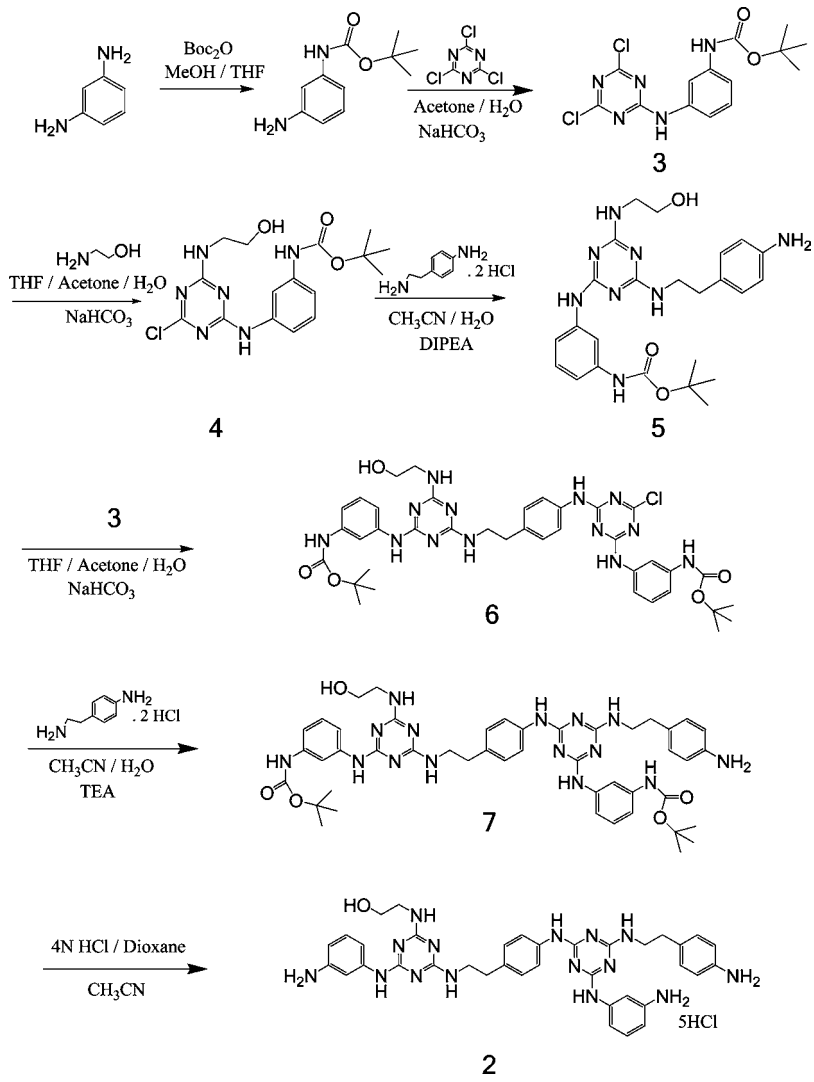
10.1021/op900202b CCC: $40.75 © 2009 American Chemical Society

Published on Web 10/01/2009

Scheme 1. **Retrosynthetic analysis of compound 7**



Scheme 2. **Gram-scale synthesis of 2**



revealed several limitations for the subsequent large-scale preparation of this compound using the chemistry detailed in Scheme 2. For example, selective monoprotection of the amine of 1,3-phenylenediamine using di-*tert*-butyldicarbonate (Boc_2O) required an excess of the diamine (6 equiv), the product yield was 15% mixed with unreacted material, and purification necessitated the use of large amounts of solvent on silica gel. The next step gave a (2:1) mixture of mono- and diaminoaniline triazine products. After purification, the yield of monosubstituted product **3** was only 45%. The third step was the introduction of the ethanolamine substituent on the triazine ring in the presence of base. This gave the expected chloro-triazine **4** in 55% yield contaminated with the corresponding diethanolamine triazine byproduct. The formation of alcohol **5** was achieved by reacting 4-aminophenethylamine with **4** in the presence of organic base. This reaction gave product that required tedious purification due to contaminants (1−5%) present in the aminophenethylamine, including oxidized amines. This resulted in

inconsistent product yields related to these impurities which were carried downstream and complicated the subsequent steps. The next step was reaction of triazine **3** and the alcohol derivative **5** in the presence of base to form the dimer **6**. Although the desired product was formed in 75% yield, the purity of **6** was only <95 wt % due to contaminants from the former step. All attempts to crystallize the product did not remove the impurities. The chloro-triazine **6** was then reacted with 4-aminophenethylamine and triethylamine to afford the penultimate **7**. Again, the purification of this product was difficult, and the yield (58%) and purity (<85% weight %) were not acceptable. The last step was deprotection of the Boc groups using HCl/dioxane to give the salt **2**. In order to remove remaining impurities, particularly the residual oxidized amine sideproducts, **2** was suspended in methanol and heated at reflux for several hours. This gave the final product **2** in 69% yield and <97 wt % % purity.

However, this procedure was sufficient to produce six batches of 0.04−0.5 g of product in overall yields of 10−15% and 85−95 area % purity. The following safety, technical, and operational issues were identified during this phase. The process (1) gave low yields of moderate-purity product and high waste loads, (2) entailed long cycle times, (3) required column chromatography, (4) involved excessive handling operations, (5) contained several operations of concentrations to dryness, and (6) required the use of high-quality aminophenethylamine, a relatively expensive reagent.

Process Development

To address these aforementioned issues, process development was performed prior to further triazine **2** scale-up. Although 3-(*tert*-butoxycarbonylamino)aniline is a commercial product, it is not readily available, and is relatively expensive. We hoped we could prepare this from 1,3-phenylenediamine and avoid an expensive starting material. For the scale-up of compound **2**, the best result was obtained when Boc_2O was dissolved in tetrahydrofuran and was added slowly to a solution of phenylenediamine in methanol over one hour at −10 °C. Under these conditions, the excess of 1,3-phenylenediamine could be reduced from six to one equivalent which permitted facile purification of the final product. The workup consisted of solvent exchange to first tetrahydrofuran and then dichloromethane, a simple filtration of the crude mixture (consisting of product, traces of unreacted 1,3-phenylenediamine and oxidized amines) on a silica gel pad, and recrystallization to produce the protected 1,3-phenylenediamine in 64−65% yield. The product yield of this step was increased over 4-fold, and the volume of solvents used was reduced by 80%.

The next step was preparation of monosubstituted triazine **3**. It has been reported[2a] that aniline reacted with cyanuric chloride in the presence of bicarbonate at 0−5 °C (pH = 5−7) and gave 2-anilino-4,6-dichlorotriazine. In our hands, this reaction tended to be problematic with regards to control of the temperature, pH, and product yield (<50%) and gave a (2:1) mixture of mono- and dianilino-triazine products. To address these issues, the addition rate of aniline was reduced to ∼2 h, and the base (saturated sodium bicarbonate) was added only at the end of the reaction to minimize competition between nucleophilic substitution by the amine and base-catalyzed hydrolyses of the chlorine atom. This gave high yields (>97%) of triazine **3**. Better results were also obtained by lowering the temperature of the reaction from 0−5 to −10 °C. These two changes avoided the formation of the di(Boc-aminoaniline) side product and exclusively gave the triazine **3** in high yield (99%) and high purity (>99.7%).

Further improvements were achieved in the third step by slowing the addition rate of ethanolamine to triazine **3** to a total of 30 min at 0−5 °C. Again, the rate, the order of addition of the reactants, as well as the control of the internal reaction temperature played important roles for the achievement of high yields of triazine **4**. The internal reaction temperature was maintained ≤10 °C to avoid the formation of the diethanolamine triazine byproduct. In order to increase the yield, saturated sodium bicarbonate was added last to the reaction mixture. Chloro-triazine **4** was subsequently isolated in 90% yield after workup by precipitation from TBME and heptane.

The third substitution on the triazine ring required the addition of 4-aminophenethylamine. Immediate improvement was achieved by the use of high purity (99.9%) 4-aminophenethylamine hydrochloride in place of the free base which contained numerous impurities. The hydrochloride salt is a stable compound and easy to handle compared to the free base. The salt was first added portionwise over 15 min, followed by DIPEA, to a solution of chloro-triazine **4** in aqueous acetone. A 2-fold excess of the salt was necessary to avoid formation of the corresponding cross-linked side product formed between chloro-triazine **4** and product **5**. This modified procedure produced the alcohol **5**, isolated by precipitation from methylene chloride in 83% yield.

In order to avoid the displacement of both chlorine atoms in **3** by the 4-aminophenethylamine derivative **5**, various conditions were examined including varying the addition rate of **3**, the reaction temperature, and stoichiometry. The best result for the formation of triazine **6** was achieved when both **3** and **5** were used in equimolar ratio. Thus, to a solution of alcohol **5** in tetrahydrofuran was added **3** in acetone/water over 10 min followed by the addition of saturated sodium bicarbonate. After workup and crystallization from ethyl acetate/heptane, compound **6** was obtained in 94% yield and 99.9% area % purity. The results were reasonably consistent from batch to batch on a 0.5 kg scale.

In the penultimate step, initial attempts using equimolar amounts of **6** and 4-aminophenethylamine hydrochloride in the presence of sodium bicarbonate failed to afford a good yield of **7**. On the other hand, using portionwise addition of a 2-fold excess (to accelerate the reaction) of 4-aminophenethylamine hydrochloride followed by triethylamine, triazine **7** was produced in 97% yield and 99.9 area % purity after refluxing for one day. The excess of 4-aminophenethylamine was easily removed by acidic extraction during workup. On a 0.7 kg scale, a quantitative yield of product **7** was obtained. Deprotection of the Boc group using 4 N HCl/dioxane produced the API, which after trituration gave salt **2** in high purity (>99.9%) and high yield (90%).

This improved process produced 15 < 30 g batches, 5 × 250 g batches, and one 450 g batch of product **2** in an overall

yield of 62% starting from protected 3-aminoaniline. Development is in progress, and we anticipate to incorporate further improvements in the upcoming larger scale campaign.

Conclusions

A practical large-scale procedure has been developed for the preparation of *N*,*N*′-bis(4,6-disubstituted 1,3,5-triazin-2-yl)-4-aminophenethylamine **2**. The optimized seven-step procedure uses inexpensive starting materials and eliminates several nonscalable operations, and nonprocess-friendly solvents such as ether and hexanes are no longer required. The process is now well suited for further scale-up. As a result of this improved synthesis, the overall product yield increased from 7% to 62% (starting from protected 3-aminoaniline). Also, the total solvent volumes were significantly reduced: organics by 70%, and aqueous by 20%, although there is still significant latitude for further solvent reduction. The compound **2** is currently under evaluation by Laboratorios Dermatologicos, Mexico, as part of a development program that targets novel drugs for the treatment of dermatological diseases.

Experimental Section

All HPLC and mass spectra were recorded on a HP 1100 LC−MS Agilent instrument using a diode array detector at 210 and 254 nm. An analytical C18 column (75 mm × 4.6 mm, 5 μm) with a gradient of 15−99% acetonitrile/water containing 0.01% TFA and a flow of 2 mL/min (temperature 30 °C) was used.

3-(*tert*-Butoxycarbonylamino)aniline. 1,3-Phenylenediamine (400.0 g, 3.7 mol) was dissolved in methanol (16 L) at −10 °C. A solution of Boc_2O (768.0 g, 3.52 mol) in THF (4 L) was added over 24 h at −10 °C ± 4 °C. The mixture was then stirred at room temperature for 20 h, diluted with THF (500 mL), and concentrated under vacuum to 2 L. The dark-brown solution was coevaporated with THF (5 × 1 L) and with dichloromethane (5 × 1 L) and then diluted to 3.5 L with dichloromethane. The solution was filtered through a silica gel pad (20 × 30 cm^2) and eluted with 35% ethyl acetate/heptane in order to remove a black contaminant. The filtrate was concentrated in vacuo to 1.0 L. The yellow solution was coevaporated with TBME (5 × 1 L), diluted to 1.6 L with TBME, and crystallized by the addition of heptane (6.6 L). The material was collected by filtration, washed with heptane (2 × 1 L), and dried under vacuum to yield a white solid (473.26 g, 64%). Mp = 94−95 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.12 (s, 1H); 7.06 (t, J = 8.0 Hz, 1H); 6.91 (d, J = 8.2 Hz, 1H); 6.80 (s, 1H); 6.63 (d, J = 7.8 Hz, 1H); 5.96 (broad, 2H); 1.47 (s, 9H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ:153.02; 146.28; 139.64; 129.96; 110.67; 109.61; 105.99; 80.66; 28.58 (3 carbons overlapped); LRMS (ESI): *m*/*z* 209 (MH^+), 231 (MNa^+); HPLC: t_R = 1.7 min (purity 100% at 210 and 254 nm).

2-[3-(*tert*-Butoxycarbonylamino)phenylamino]-4,6-dichloro-1,3,5-triazine (3). Acetone (3.5 L) and water (3.5 L) were stirred vigorously and cooled to −10 °C. Cyanuric chloride (442.1 g, 2.40 mol) was added portionwise over 15 min to obtain a homogeneous suspension. A solution of mono-Boc-1,3-phenylenediamine (499.3 g, 2.4 mol) in acetone (3.5 L) was added dropwise over 2 h, keeping the temperature between −5 to −10 °C. A white slurry was obtained. This material was stirred at room temperature for 60 min (pH = 2) and then basified to pH 8 by pouring onto a saturated solution of sodium bicarbonate (10 L). The mixture was stirred for 30 min. The precipitate was filtered, washed with water (5 × 4 L), and dried under vacuum for 2 d to yield an amorphous white solid (850.3 g, 99.6%). Mp = 172 − 173 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.69−7.73 (m, 1H); 7.14−7.30 (m, 3H); 1.52 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 170.32; 169.42; 164.52; 153.39; 140.72; 137.71; 129.53; 116.47; 115.78; 112.55; 79.78; 28.78 (3 carbons overlapped); LRMS (ESI): *m*/*z* 358 (MH^+); HPLC: t_R = 4.5 min (purity >99.2% at 210 nm and >99.3% at 254 nm).

2-(4-(3-(*tert*-Butoxycarbonylamino)phenylamino)-6-chloro-1,3,5-triazin-2-ylamino)ethanol (4). The triazine **3** (835.0 g, 2.35 mol) was dissolved in THF (11.7 L) and cooled to 0−5 °C. A solution of ethanolamine (157.5 g, 2.59 mol) in acetone (3.3 L) and water (3.3 L) was added portion-wise over 30 min while maintaining internal temperature at 1−8 °C. A saturated solution of sodium bicarbonate (668.0 mL) was then added in one portion to the light-yellow mixture. The solution was stirred at room temperature for 20 h, concentrated under vacuum to 3 L, and coevaporated with ethyl acetate (3 × 2 L). The solution was diluted with ethyl acetate (6.7 L) and washed with water and brine, dried over magnesium sulfate−charcoal−silica gel for removal of unreacted starting material and color, filtered, and concentrated to 1.3 L. The yellow solution was diluted with TBME to 3.3 L and added dropwise (1 h) under nitrogen to heptane (22.5 L). This produced a white solid which was filtered, washed with heptane (2 × 800 mL), and dried. It was then triturated with TBME/heptane (2:1 solution, 1.3 L), filtered, and dried under vacuum to yield a white, amorphous solid (798.33 g, 89.5%). Mp = 157−159 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ: 8.2 (broad, 1H); 7.76 (broad, 1H); 7.24−7.30 (m, 1H); 7.08−7.22 (m, 3H); 6.96 (broad, 1H); 3.72 (t, J = 5.6 Hz, 1H); 3.67 (t, J = 5.6 Hz, 1H); 3.61 (broad, 1H); 3.46−3.52 (m, 1H); 1.51 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 169.37; 168.56; 166.36; 166.19; 164.12; 163.93; 154.06; 153.98; 139.85; 139.76; 139.62; 139.24; 139.15; 128.92; 128.68; 128.62; 115.23; 114.76; 113.88; 111.36; 111.03; 79.77; 79.64; 65.75; 60.51; 60.38; 43.28; 43.04; 27.79; 27.60 (3 carbons overlapped); (mixture of rotamers); LRMS (ESI): *m*/*z* 381 (MH^+), 403 (MNa^+); HPLC: t_R = 3.3 min (purity 97% at 210 and 254 nm).

Diethanolamine triazine byproduct (2-(4-(3-(*tert*-butoxycarbonylamino)phenylamino)-6-(3-(*tert*-butoxycarbonylamino)phenylamino-1,3,5-triazin-2-ylamino)ethanol): ^{1}H NMR (DMSO-d_6, 400 MHz) δ: 10.12 (broad, 2H); 9.21 (broad, 2H); 7.40−7.55 (m, 2H); 7.22−7.40 (m, 1H) 7.04−7.25 (m, 5H); 1.52 (s, 18H); LRMS (ESI): *m*/*z* 529 (MH^+), 551 (MNa^+); HPLC: t_R = 5.0 min.

2-(4-(4-Aminophenethylamino)-6-(3-(*tert*-butoxycarbonylamino)phenylamino)-1,3,5-triazin-2-ylamino)ethanol (5). The chloro-triazine **4** (790.0 g, 2.07 mol) was dissolved in a mixture of acetonitrile (15.8 L) and water (316 mL). 4-Aminophenethylamine hydrochloride (862.82 g, 4.15 mol) was added portionwise, followed by DIPEA (1.07 kg, 8.29 mol). The mixture was then heated at 60 °C under nitrogen for 48 h. The mixture was cooled and filtered on fiberglass. The filtrate

was concentrated to 3 L, diluted with water (3 L), and concentrated again to 3 L to remove acetonitrile. This solution was diluted to 5 L with water and extracted with ethyl acetate (7.4 L). The organic layer was separated and washed with brine and then extracted with 2 N hydrochloric acid (5.5 L). This acidic phase was washed with ethyl acetate and basified to pH 8 with solid sodium bicarbonate. A residue was obtained which was redissolved in ethyl acetate (7.3 L), washed with brine, treated with magnesium sulfate−charcoal, filtered on Celite, and concentrated to 2.8 L. This mixture was added dropwise to heptane (18 L) under nitrogen. A gummy, brown solid was obtained and separated by decantation. Methylene chloride (3 L) was added, and the resulting mixture was slowly stirred overnight at 20−25 °C. The fluffy solid was filtered off, washed with methylene chloride (1.5 L), and dried under vacuum to yield a beige amorphous solid (826.5 g, 83%). Mp = 154−155 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.26 (broad, 1H); 7.13 (t, J = 7.8 Hz, 1 H); 6.98 (d, J = 8.2 Hz, 4H); 6.68 (d, J = 2.5 Hz, 2H); 3.68 (broad, 2 H); 3.52 (broad, 4H); 2.72 (t, J = 7.4 Hz, 2H); 1.49 (s, 9H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 154.06; 145.36; 139.61; 129.47; 129.31 (3 carbons overlapped); 128.52; 115.85 (4 carbons overlapped); 115.21; 114.57; 112.81; 110.90; 79.54; 61.32; 42.83; 42.43; 35.11; 27.55 (3 carbons overlapped); LRMS (ESI): m/z 481 (MH^+), 503 (MNa^+); HPLC: t_R = 1.8 min (purity 100% at 210 and 254 nm).

2-(4-(3-(*tert*-Butoxycarbonylamino)phenylamino)-6-(4-(4-(3-(*tert*-butoxycarbonylamino)phenylamino)-6-chloro-1,3,5-triazin-2-ylamino)phenethylamino)-1,3,5-triazin-2-ylamino)-ethanol (6). The alcohol **5** (820.0 g, 1.71 mol) was dissolved in THF (10.9 L), and a solution of triazine **3** (607.7 g, 1.71 mol) in acetone (2.9 L) and water (2.9 L) was added over 10 min followed by a saturated solution of sodium bicarbonate (2.9 L). The mixture was stirred at 20−25 °C for 24 h and then concentrated to 5 L to remove volatiles. The solution was diluted with water (2 L) and concentrated to 4 L. The brown solution was then extracted with ethyl acetate (9.0 L), washed with water and brine, and treated with magnesium sulfate−charcoal. The solution was then filtered and concentrated to 3.0 L. This mixture was added dropwise under nitrogen to heptane (18.0 L) with stirring. The precipitated beige solid was filtered, washed with heptane (2 × 1 L), and dried under vacuum to yield a beige amorphous solid (1.29 kg; 94.7%). Mp = 146−149 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.75−7.72 (m, 3H); 6.88−7.32 (m, 9H); 3.68 (broad, 2H); 3.60 (broad, 2H); 3.54 (broad, 2H); 2.84 (t, J = 7.2 Hz, 2H); 1.49 (s, 18H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 171.83; 165.94; 164.52 (2 carbons overlapped); 154.02; 140.50; 139.55 (2 carbons overlapped); 136.57; 135.22; 128.96 (3 carbons overlapped); 128.71 (3 carbons overlapped); 128.50; 120.94 (3 carbons overlapped); 114.54; 112.85 (2 carbons overlapped); 110.90; 79.85; 79.57; 61.35; 60.38; 42.85; 42.11; 35.35; 27.59 (3 carbons overlapped); 27.58 (3 carbons overlapped);19.71; 13.30; LRMS (ESI): m/z 800 (MH^+), 822 (MNa^+); HPLC: t_R = 3.3 min (purity 100% at 210 and 254 nm).

2-(4-(4-(4-(4-Aminophenethylamino)-6-(3-(*tert*-butoxycarbonylamino)phenylamino)-1,3,5-triazin-2-ylamino)phenylethylamino)-6-(3-(*tert*-butoxycarbonylamino)phenylamino)-1,3,5-triazin-2-ylamino)ethanol (7). The triazine **6** (1.1 kg, 1.39 mol) in acetonitrile (15.5 L) and water (465 mL) was treated with 4-aminophenethylamine hydrochloride (0.8 kg, 3.84 mol) portionwise, followed by the addition of triethylamine (0.7 kg, 6.82 mol). The mixture was then heated at 60 °C for 24 h. The solvent was concentrated under vacuum to 3.0 L, coevaporated with ethyl acetate (3 × 2 L), and diluted with ethyl acetate to 8.0 L. The solution was washed with water and brine, treated with magnesium sulfate−charcoal, and filtered. The filtrate was acidified with 2 N hydrochloric acid (1.5 L) to afford a brown, oily residue which was separated with the aqueous phase from the organic layer. The aqueous mixture was basified to pH 8 with solid sodium bicarbonate. The solid was separated, dissolved in ethyl acetate (9 L), treated with magnesium sulfate−charcoal, filtered, and concentrated to 3.5 L under vacuum. This solution was added dropwise to heptane (18.6 L) to form a solid. The precipitate was filtered, washed with heptane (2 × 750 mL), and dried under vacuum to yield a beige amorphous solid (1.24 kg, 97%). Mp = 104−107 °C; ^{1}H NMR (CD_3OD, 400 MHz) δ: 7.48−8.14 (m, 4H); 7.06−7.34 (m, 7H); 6.90−7.06 (m, 3H); 6.66 (d, J = 8.2 Hz, 2H); 3.68 (broad, 2H); 3.53 (broad, 6H); 2.82 (broad, 2H); 2.74 (t, J = 7.6 Hz, 2H); 1.48 (s,18H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 171.13; 166.07; 165.99; 165.63; 165.00; 164.66; 164.33; 154.08; 154.02; 145.14; 140.43; 140.19; 139.60; 139.51; 138.11; 137.73; 133.91; 133.53; 129.60; 129.47; 129.35 (2 carbons overlapped); 129.23; 128.82 (2 carbons overlapped); 128.58; 120.89; 120.27; 115.94 (2 carbons overlapped); 115.05; 114.67; 112.95; 111.31; 110.93; 79.61; 61.30; 60.38; 42.91; 42.56; 42.20; 36.25; 35.15; 34.67; 34.60; 34.56; 34.51; 31.58; 27.60 (6 carbons overlapped); 27.59 (3 carbons overlapped); 25.13; 25.03; 22.53; 21.83; 19.86; 19.76; 19.71; 17.98; 13.30; 13.29; 10.62. (mixture of rotamers); LRMS (ESI): m/z 901 (MH^+) 923 (MNa^+); HPLC: t_R = 2.7 min (purity 100% at 210 and 254 nm).

2-(4-(4-(4-(4-Aminophenethylamino)-6-(3-aminophenylamino)-1,3,5-triazin-2-ylamino)phenylethylamino)-6-(3-aminophenylamino)-1,3,5-triazin-2-ylamino)ethanol pentahydrochloride salt (2). The protected triazine **7** (1.24 kg, 0.7 mol) was dissolved in a mixture of 4 N hydrochloric acid/dioxane (7.9 L) and water (0.73 L). This mixture was stirred at 20−25 °C for 60 min, diluted with acetonitrile (23 L), and stirred 15 min to afford a beige solid. The material was filtered and washed with methanol (12 L), tetrahydrofuran (6 L), and TBME (6 L) and then dried for 24 h. The solid was dissolved in water (58 L). The clear, pale-yellow solution was filtered on a 0.22 μm membrane, frozen and lyophilized. A light beige fluffy solid was obtained (1.11 kg, 90%). Mp = 220−222 °C; ^{1}H NMR (D_2O, 400 MHz) δ: 6.80−7.80 (m, 16H); 3.00−3.70 (m, 8H); 2.60 (broad, 4H); ^{13}C NMR (CD_3OD, 100 MHz) δ: 161.27; 155.04; 154.22; 151.99; 139.28; 137.88; 137.48; 135.47; 134.27; 130.26 (8 carbons overlapped); 128.92 (3 carbons overlapped); 128.36; 123.14 (3 carbons overlapped); 120.17; 118.91; 114.64 (2 carbons overlapped); 59.53; 42.95; 42.43 (2 carbons overlapped); 34.28 (2 carbons overlapped); LRMS (ESI): m/z 700 (MH^+) 350 (($M + 2 H^+$)/2); HPLC: t_R = 3.6 min (purity 100% at 210 and 254 nm).

Acknowledgment

We thank Shaun D. Abbott for reading the manuscript and Joël Lechasseur for technical assistance.

Received for review August 3, 2009.

OP900202B