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Synthesis of Patent Blue derivatized hydrophilic dendrons dedicated to sentinel node detection in breast cancer

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ABSTRACT

In the last decade, methods for the precise localization of sentinel lymph node (SLN) have drawn tremendous attention by oncology surgeons and researchers in the field of medical diagnosis. The accurate identification and characterization of lymph nodes by imaging has important therapeutic and prognostic significance in patients with newly diagnosed cancers. The SLN is the first lymph node that receives lymphatic drainage from the site of a primary tumor. Two biocompatible dendronized phosphonates, one bearing a Patent Blue (PB VF) dye at its periphery, where synthesized. Indeed, such a blue dye is currently injected to label the lymph node system for its per-operative detection. Therefore, developing chemistry of Patent Blue VF to optimize early diagnosis is of great current interest.

1. Introduction

In the field of cancer research, the term sentinel lymph node (SLN) refers to any node receiving lymph drainage from the tumor site and containing most likely malignancy if the tumor has metastasized. The treatment of breast cancer through SLN detection is currently a great challenge.[1] Indeed, an early detection of this node can prevent patients with a low risk of lymph node involvement from suffering an unnecessary axillary lymphadenectomy.[2] Dyes have been exploited in medicine for more than one century to explore the lymphatic system. Several dyes more or less toxic were used, such as Gerota mass (Prussian blue and iron ferrocyanide in oil), cresyl blue (amino-dimethylamino ethyldiphenazonium chloride; $C_{17}H_{20}N_3OCl$) or indigo carmine (indigo tin disulfonate sodium) in pre-mortem, then Patent Blue V or Evans blue during a surgery.[3] These latter ones have been then replaced by fluorescent substances, Indian ink or activated carbon.[4] The next step has been to use radioactive gold colloids (Au 198) in diagnosis as well as in therapy of breast cancer, then replaced by colloids labeled with ^{99m}Tc.[5] Most medical teams using sentinel node biopsy in the treatment of breast cancer inject either a radioactive colloid (^{99m}Tc-labeled RuS)[6] and/or a blue dye[7] to label the lymph node system for its per-operative detection. Such procedure, involving then two injections, is based on a gamma probe and/or visual color detections, respectively. If radioisotope injection is widely used today in the most advanced countries, new strategies using non-nuclear detection are also investigated, and in this context, we decided to develop a new and original project based on dendritic magnetic iron oxide nanoparticles covalently connected to a blue dye (PB VF) for magneto-optical detection. Indeed, in the field of biology and medicine, dendrimers and their elementary unit called dendron, are showing great promise as they combine unique advantages such as monodispersity although they can be polyfunctional, easy control of their physico-chemical characteristics as a function of their generation, tuneable amphiphilicity (to cross biological barriers).[8] In our team, which has been interested for some years in surface engineering of super paramagnetic iron oxide (SPIO) NPs dedicated to MRI contrast enhancement,[9] new attempts to develop original dendronized NPs have recently been investigated in the field of cancer diagnosis.

2. Results and discussion

Here, we present the synthetic route to two new dendronized phosphonates: **1** which is bearing a primary alcohol function at its periphery, and **2**, bearing a Patent Blue dye dedicated to SLN detection (Scheme 1). Key-functionalities have to appear in the dendritic structure: (i) a coupling agent at the focal point to ensure a strong and stable connection to the surface of SPIO NPs; (ii) oligoethyleneglycol chains to reach hydrophilicity and confer biocompatibility to the final nano-object; (iii) in the case of **2**, a blue dye for optical detection.

Dealing with the coupling agent, phosphonates are candidate of choice as former studies pointed out a stronger binding and a higher grafting rate than with carboxylate anchors.[10] Moreover,

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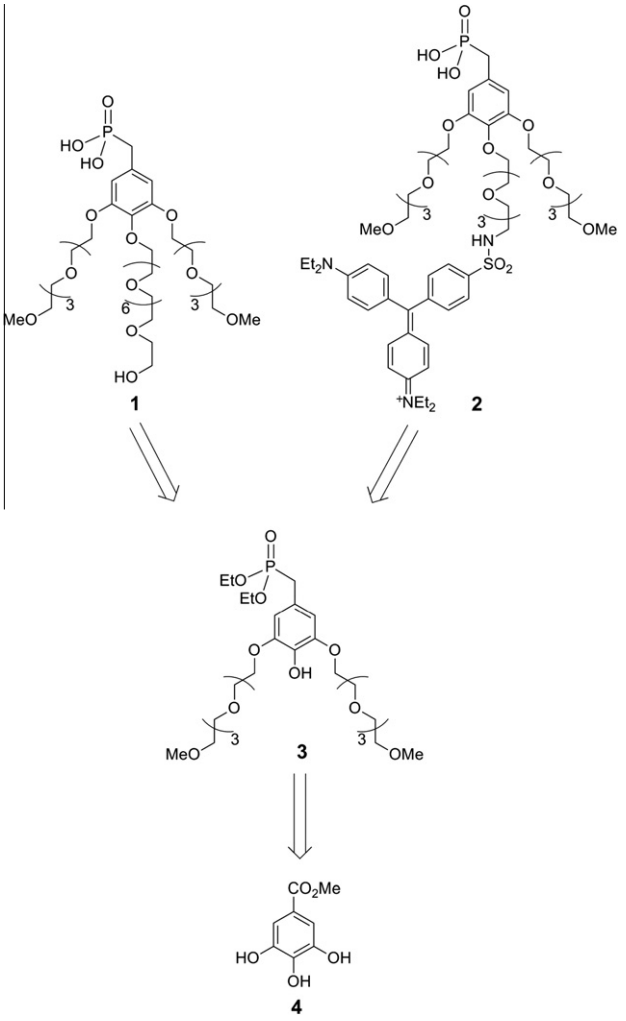
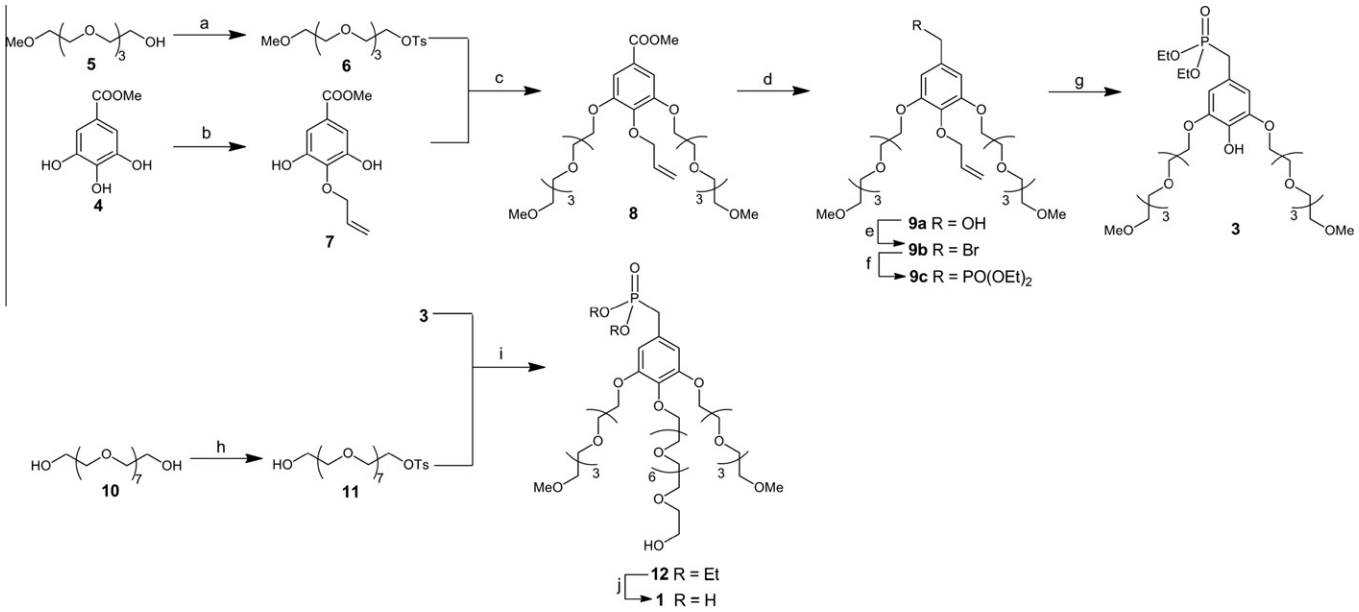


Scheme 1. Retrosyntheses to dendritic phosphonates **1** and **2**.

phosphonates are known to stabilize suspensions in water at physiological pH and to preserve the magnetic properties.[9,10] Compounds 1 and **2** can be obtained from key building block **3** which was synthesized from methyl gallate **4** in seven steps (Scheme 2): *para*-allylated methyl gallate **7** and tosylated tetraethyleneglycol monomethyl ether **6** can easily be produced in good yields from commercially available compounds **4** and **5,** respectively, following described one-step-procedures.[11,12] Then, a Williamson reaction in acetone at 60 °C in the presence of K_2CO_3 and KI (catalytic) allows obtaining ester **8** in 70% yield. From here, the allylated ethyl phosphonate **9c** can be obtained thanks to a three-step sequence: (i) reduction of the ester function using $LiAlH_4$ as reducing agent (obtention of the benzyl alcohol **9a**, 97%), (ii) the treatment with PBr_3 (obtention of the benzyl bromide **9b**, 99%) and (iii) 2-hour refluxing of **9b** at 160 °C in $P(OEt)_3$ (obtention of the allylated ethyl phosphonate **9c**, 85%). Finally, deallylation by the treatment with $Pd(PPh_3)_4/NaBH_4$ leads to key phosphonate **3** in 90% yield. In order to prepare **1**, a monotosylation of commercially available octaethyleneglycol **10** was first achieved to obtain **11** in 59% yield.[13] Then, **11** was subjected to etherification with **3** to yield ethyl phosphonate **12** (75%) which was finally converted into its corresponding phosphonic acid **1** in a good 93% yield by a silylation/methanolyse step using an excess of bromotrimethylsilane.[14]

Concerning the design of a blue dye-derivatized dendritic phosphonate, our first idea was to use Patent Blue V **13** (Scheme 3), which is one of the most used Vital Blue dye in sentinel node detection.[15] Retrosynthetically, the considered strategy was the elaboration of a Patent Blue V-derivatized dendron **14** from etherification between **3** and **15**. Unfortunately, our attempts to obtain such an oligoethyleneglycol chain linked to the Patent Blue V dye failed (Table 1): neither a Williamson reaction between **13** and **16a** (entry 1), nor a Mitsonobu reaction between **13** and **16b** (entry 2) succeeded and compound **17** was never isolated. In the first attempt (entry 1) conversion was very low and a hard-to-purify mixture of dye-derivatives was obtained, whereas, in the second investigation (entry 2) no conversion was observed, even after several days of reaction.

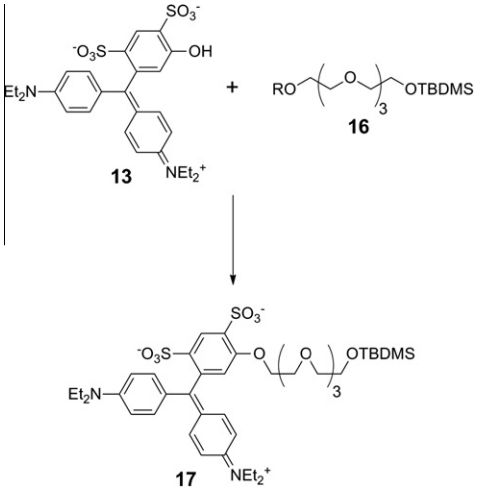


Scheme 2. Synthesis of key-synthon **3** and final steps to dendron **1**. Reagents and conditions: (a) *p*TsCl (1.1 equiv), NaOH (1.7 equiv), THF/H_2O, 0 °C→rt, one night, 96%; (b) allylbromide (1.0 equiv), $KHCO_3$ (4.0 equiv), KI (0.005 equiv), DMF, 30 °C, 5 days, 85%; (c) **2** (1.0 equiv), **1** (2.2 equiv), K_2CO_3 (5.0 equiv), KI (0.3 equiv), acetone, 60 °C, 24 h, 70%; (d) $LiAlH_4$ (0.5 equiv), THF, 0 °C→rt, 1 h, 97%; (e) PBr_3 (1.8 equiv), CH_2Cl_2, 0 °C→rt, 2 h, 99%; (f) $P(OEt)_3$, 160 °C, 2 h, 85%; (g) $Pd(PPh_3)_4$ (0.02 equiv), $NaBH_4$ (2.5 equiv), THF/DMF, rt, 2 h, 90%; (h) *p*TsCl (1.2 equiv), Ag_2O (1.8 equiv), CH_2Cl_2, 0 °C, 15 min, 59%; (i) **3** (1.0 equiv), **11** (1.0 equiv), K_2CO_3 (2.5 equiv), KI (0.3 equiv), acetone, 60 °C, 24 h, 75%; (j) TMSBr (15.0 equiv), CH_2Cl_2, 0 °C→rt, one night, 93%.

These results, together with the fact that we were not able to find any described reaction involving **13** as a reactant or reagent, lead us to the conclusion that the phenol function of **13** is not reactive enough, certainly due to mesomeric stabilization effect of the sulfonate functional groups in *ortho* and *para* positions. Consequently, we decided to look for a new alternative and work with Patent Blue VF **18** (Scheme 4), which has a structure similar to the Patent Blue V, but without a phenol function on its aromatic system. Indeed, there is the possibility to regioselectively activate the less hindered sulfonate function of **18** into its corresponding sulfonyl chloride, which can then react with an amine to form a sulfonamide link.[16] It should be noted that the activation of Patent Blue V **13** into one of its sulfonyl chloride was also tested but it seemed that in this case the activation was not regioselective at all and that the presence of the unprotected phenol led to secondary reactions. In order to reach Patent Blue VF-dye derivatized phosphonic acid **2**, amine-terminated dendron **22** was first prepared from key-synthon **3** and commercially available amino tetraethyleneglycol **19** in 4 steps: a Boc protection of the amine function followed by a tosylation of the alcohol function led to **20** in 80% overall yield. Etherification between **20** and **3** allowed to obtain **21** (95% yield) which was treated with an excess of trifluoroacetic acid to yield **22** (97%). Sulfonyl chloride **23** was prepared from Patent Blue VF **18** following Tahtaoui et al.'s procedure.[16] Sulfonamide linkage was then achieved between **23** (used without further purification) and **22** in the presence of triethylamine and 4-DMAP and allowed obtaining dye derivatized ethyl phosphonate **24** in a moderate 54% yield. Finally **24** was converted into its corresponding phosphonic acid **2** in 95% yield by the treatment with an excess of bromotrimethylsilane.[17]

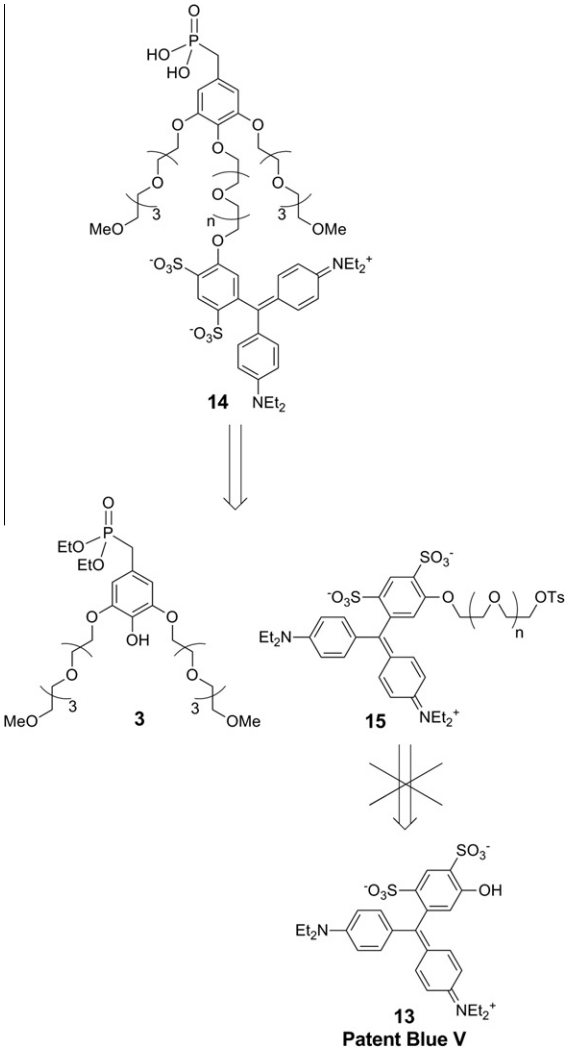
The structures and purity of all compounds were confirmed by ^{1}H and ^{13}C NMR and mass spectrometry (see Supplementary data).

Table 1
Attempts for synthesizing a Patent Blue V-derivatized oligoethyleneglycol chain **17**



Entry	Electrophile	Conditions	Yield (%)
1	**16a** R = Tos	K_2CO_3, KI, DMF, 60 °C	—[a]
2	**16b** R = H	DEAD, PPh_3, THF, rt	—[b]

[a] Hard-to-purify mixture of dye-derivatized compounds in very low yield.
[b] No conversion observed, even after long reaction times (17 days).



Scheme 3. First considered strategy to a Patent blue V-derivatized dendron.

3. Conclusion

In conclusion, we have prepared two new low-generation hydrophilic and biocompatible dendritic phosphonates **1** and **2**, either derivatized with a primary alcohol allowing further grafting of biological effectors (for efficient targeted imaging and therapy), or with a Vital Blue dye ensuring optical detection. The functionalization of 10 nm iron oxide nanoparticles with these original coatings is currently under investigation in our laboratory in order to develop highly efficient and stable (in vivo) magneto-optical probes (Fig. 1).

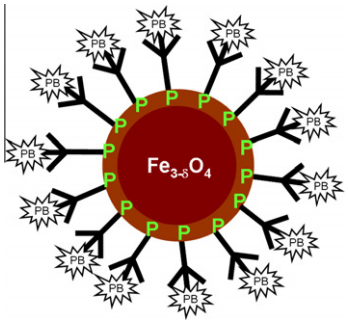
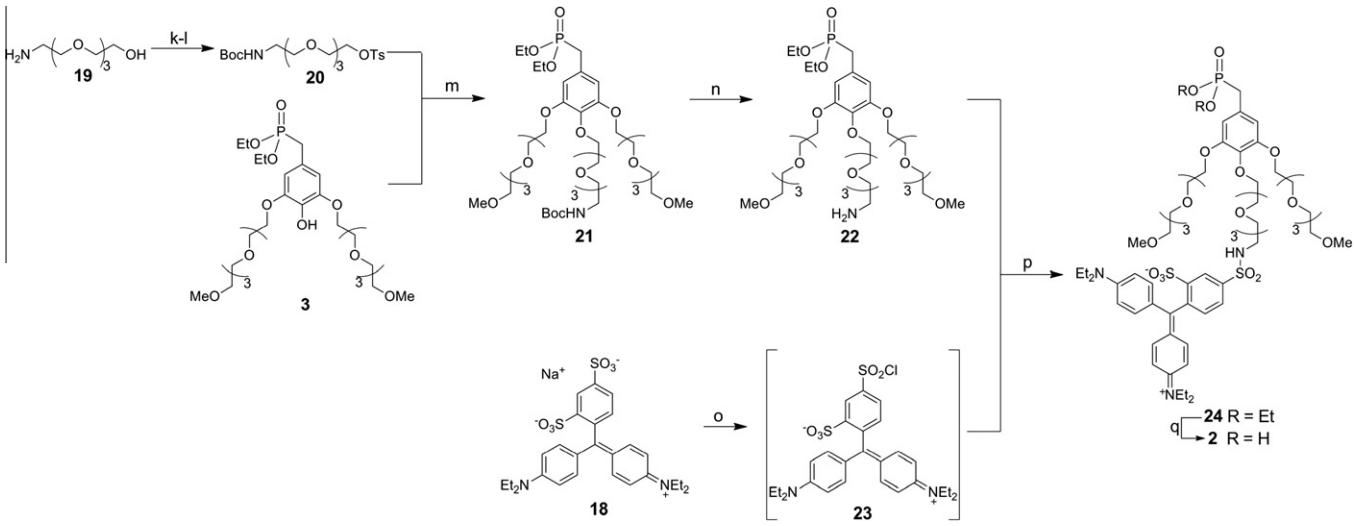


Figure 1. Patent Blue (PB) derivatized dendronized SPIO NPs.



Scheme 4. Final steps to dye-derivatized dendron **2**. Reagents and conditions: (k) Boc_2O (1.0 equiv), CH_2Cl_2, 0 °C→rt, one night, 86%; (l) *p*TsCl (1.1 equiv), NEt_3 (2.3 equiv), CH_2Cl_2, 0 °C→rt, 40 h, 93%; (m) **3** (1.0 equiv), **20** (1.0 equiv), K_2CO_3 (4.0 equiv), KI (0.3 equiv), acetone, 60 °C, 48 h, 95%; (n) TFA (15.0 equiv), CH_2Cl_2, 0 °C→rt, one night, 97%; (o) $POCl_3$ (20.0 equiv), rt, 72 h; (p) **22** (1.0 equiv), **23** (1.0 equiv), NEt_3 (3.0 equiv), 4-DMAP (0.1 equiv), CH_2Cl_2/DMF 4:1, 0 °C→rt, one night, 54% (steps n + o); (q) TMSBr (15.0 equiv), CH_2Cl_2, 0 °C→rt, one night, 95%.

Acknowledgments

We would like to thank Emilie Voirin and Emilie Couzigné for their assistance. We also thank the European Community for funding through the NANOMAGDYE project CP-FP 214032-2 and Dr. Geneviève Pourroy, coordinator of the NANOMAGDYE project.

Supplementary data

Supplementary data (synthetic procedures, characterization and ^1H and ^{13}C NMR spectra for compounds **1**, **2**, **3, 12, 21, 22**, and **24**) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2011.03.144.

References and notes

1. Brettes, J.-P.; Mathelin, C.; Gairard, B.; Bellocq, J.-P. *Cancer du sein*, **2007**, Masson Editions.
2. (a) Cox, C. E.; Salud, C.; Whitehead, G. F.; Reintgen, D. S. *Breast Cancer* **2000**, *7*, 389–397; (b) Cody, H. S.; Fey, J.; Akhurst, T.; Fazzari, M.; Mazumdar, M.; Yeung, H. *Ann. Surg. Oncol.* **2001**, *8*, 13–19.
3. Masannat, Y.; Shenoy, H.; Speirs, V.; Handy, A.; Horgan, K. *Eur. J. Surg. Oncol.* **2006**, *32*, 381–384.
4. Nieweg, O. E. *Ann. Surg. Oncol.* **2001**, *8*, 71S–73S.
5. Jain, R.; Dandekar, P.; Patravale, V. *J. Control. Release* **2009**, *138*, 90–102.
6. Krag, D. N.; Weaver, D. L.; Alex, J. C.; Fairbank, J. T. *Surg. Oncol.* **1993**, *2*, 335–340.
7. Giuliano, A. E.; Kirgan, D. M.; Guenther, J. M.; Morton, D. M. *Ann. Surg.* **1994**, *220*, 391–401.
8. (a) Kim, M.; Chen, Y.; Liu, Y.; Peng, X. *Adv. Mater.* **2005**, *17*, 1429–1432; (b) Lartigue, L.; Oumzil, K.; Guari, Y.; Larionova, J.; Guérin, C.; Montero, J.-L.; Barragan-Montero, V.; Sangregorio, C.; Caneschi, A.; Innocenti, C.; Kalaivani, T.; Arosio, P.; Lascialfari, A. *Org. Lett.* **2009**, *11*, 2992–2995; (c) Hofmann, A.; Graf, C.; Kung, S.-H.; Kim, M.; Peng, X.; El-Aama, R.; Rühl, E. *Synthesis* **2010**, *42*, 1150–1158; (d) Hofmann, A.; Thierbach, S.; Semisch, A.; Hartwig, A.; Taupitz, M.; Rühl, E.; Graf, C. *J. Mater. Chem.* **2010**, *20*, 7842–7853.
9. (a) Daou, T. J.; Pourroy, G.; Greneche, J. M.; Bertin, A.; Felder-Flesch, D.; Begin-Colin, S. *Dalton Trans.* **2009**, *23*, 4442–4449; (b) Basly, B.; Felder-Flesch, D.; Perriat, P.; Billotey, C.; Taleb, J.; Pourroy, G.; Begin-Colin, S. *Chem. Commun.* **2010**, *46*, 985–987; (c) Basly, B.; Felder-Flesch, D.; Perriat, P.; Pourroy, G.; Begin-Colin, S. *Contrast Media and Mol. Imaging* **2010**. doi:10.1002/cmmi.416.
10. (a) Daou, T. J.; Buathong, S.; Ung, D.; Donnio, B.; Pourroy, G.; Guillon, D.; Bégin, S. *Sens. Actuators, B* **2007**, *126*, 159–162; (b) Daou, T. J.; Begin-Colin, S.; Greneche, J. M.; Thomas, F.; Derory, A.; Bernhardt, P.; Legaré, P.; Pourroy, G. *Chem. Mater.* **2007**, *19*, 4494–4505; (c) Daou, T. J.; Greneche, J. M.; Pourroy, G.; Buathong, S.; Derory, A.; Ulhaq-Bouillet, C.; Donnio, B.; Guillon, D.; Begin-Colin, S. *Chem. Mater.* **2008**, *20*, 5869–5875; (d) Boyer, C.; Bulmus, V.; Priyanto, P.; Teoh, W. Y.; Amal, R.; Davis, T. P. *J. Mater. Chem.* **2009**, *19*, 111–123.
11. Freeman, A. W.; Chrisstoffels, L. A. J.; Fréchet, J. M. J. *J. Org. Chem.* **2000**, *65*, 7612–7617.
12. Snow, A. W.; Foos, E. E. *Synthesis* **2003**, *35*, 509–512.
13. Bouzide, A.; Sauvé, G. *Org. Lett.* **2002**, *4*, 2329–2332.
14. *Compound* **1**: to a solution of **12** (250 mg, 0.25 mmol) in 15 mL of CH_2Cl_2 at 0 °C, is added dropwise 500 μL (3.75 mmol, 15.0 equiv) of TMSBr. After one night stirring at room temperature, the volatiles are evaporated and methanol is added to the crude product and evaporated several times. Compound **1** is obtained as a dark yellow oil in 93% yield without further purification. ^1H NMR (300 MHz, CD_3OD, 20 °C): δ (ppm) = 3.08 (d, 2H, ^2J = 21.5 Hz), 3.34 (s, 6H), 3.50–3.77 (m, 52H), 3.78 (t, 2H, ^3J = 5.2 Hz), 3.84 (t, 4H, ^3J = 5.2 Hz), 4.09–4.17 (m, 6H), 6.64 (d, 2H, ^4J = 2.5 Hz). ^{13}C NMR (75 MHz, CD_3OD, 20 °C): δ (ppm) = 35.61 ($^1J_{CP}$ = 135.5 Hz), 59.07, 62.16, 69.93, 70.76, 71.26, 71.30, 71.46, 71.56, 71.66, 72.88, 73.43, 73.56, 110.38 ($^3J_{CP}$ = 5.8 Hz), 129.64 ($^2J_{CP}$ = 9.1 Hz), 138.15 ($^5J_{CP}$ = 2.5 Hz), 153.65 ($^4J_{CP}$ = 2.5 Hz). ^{31}P NMR (81 MHz, CD_3OD, 20 °C): δ (ppm) = 24.32. MALDI: calculated for $C_{41}H_{78}O_{22}P$: 953.47, obtained: 953.47; calculated for $C_{41}H_{77}NaO_{22}P$: 975.45, obtained: 975.46; calculated for $C_{41}H_{77}KO_{22}P$: 991.43, obtained: 991.43; calculated for $C_{41}H_{76}Na_2O_{22}P$: 997.44, obtained: 997.44. IR (KBr, cm^{-1}): 3409, 2875, 1590, 1511, 1443, 1352, 1249, 1106, 955, 848.
15. (a) Walker, P. M.; Hussain, M.; Humphrey, C. S. *Breast* **2002**, *11*, 343–345; (b) Patel, A.; Pain, S. J.; Britton, P.; Sinnatamby, R.; Warren, R.; Bobrow, L.; Barber, R. W.; Peters, A. M.; Purushotham, A. D. *Eur. J. Surg. Oncol.* **2004**, *30*, 918–923; (c) Masannat, Y.; Shenoy, H.; Speirs, V.; Hanby, A.; Horgan, K. *Eur. J. Surg. Oncol.* **2006**, *32*, 381–384.
16. Tahtaoui, C.; Guillier, F.; Klotz, P.; Galzi, J.-L.; Hibert, M.; Ilien, B. *J. Med. Chem.* **2005**, *48*, 7847–7859.
17. *Compound* **2**: to a solution of **24** (136 mg, 0.10 mmol) in 6 mL of CH_2Cl_2 at 0 °C, is added dropwise 200 μL (1.50 mmol, 15.0 equiv) of TMSBr. After one night stirring at room temperature, the volatiles are evaporated and methanol is added to the crude product and evaporated several times. Compound **2** is obtained as a dark blue oil in 95% yield without further purification. ^1H NMR (300 MHz, DMSO-d_6, 20 °C): δ (ppm) = 1.20 (t, ^3J = 6.7 Hz, 12H), 2.78 (d, 2H, ^2J = 21.0 Hz), 3.00 (bt, 2H, ^3J = 5.8 Hz), 3.22 (s, 6H), 3.39–3.71 (m, 50H), 3.93 (bt, 2H, ^3J = 5.2 Hz), 4.00–4.07 (m, 4H), 6.57 (s, 2H), 6.98 (m, 4H, AA′ part of an AA′BB′ system), 7.23 (d, 1H, ^3J = 8.1 Hz), 7.27 (m, 4H, BB′ part of an AA′BB′ system), 7.85 (dd, 1H, ^3J = 8.1 Hz, ^4J = 1.8 Hz), 8.03 (br s, 1H), 8.35 (d, 1H, ^4J = 1.8 Hz). ^{13}C NMR (75 MHz, DMSO-d_6, 20 °C): δ (ppm) = 12.70, 35.29 ($^1J_{CP}$ = 132.7 Hz), 42.42, 45.26, 58.02, 60.19, 68.32, 68.97, 69.12, 69.56, 69.69, 69.76, 69.81, 69.94, 71.25, 71.77, 72.30, 108.98 ($^3J_{CP}$ = 5.8 Hz), 113.27, 125.84, 125.97, 126.08, 129.46 ($^2J_{CP}$ = 8.5 Hz), 131.68, 136.01 ($^5J_{CP}$ = 3.4 Hz), 139.80, 139.99, 141.38, 148.22, 151.66 ($^4J_{CP}$ = 2.2 Hz), 154.53, 173.61. ^{31}P NMR (81 MHz, DMSO-d_6, 20 °C): δ (ppm) = 18.29. MALDI: calculated for $C_{60}H_{93}N_3O_{22}PS_2$: 1302.54, obtained: 1302.57; calculated for $C_{60}H_{92}N_3NaO_{22}PS_2$: 1324.53, obtained: 1324.55. IR (KBr, cm^{-1}): 3416, 2925, 2868, 1613, 1579, 1409, 1341, 1251, 1193, 1109, 1018, 934, 830, 705, 609. UV/Vis (in H_2O): $\lambda_{max}(\varepsilon)$ = 646 (82 800) nm (shoulder around 600 nm).