Latvian Journal of Chemistry, No 1/2, 2012, 41–48
DOI: 10.2478/v10161-012-0008-9

DEVELOPMENT OF NEW TRIAZOLYL-BASED ACIDIC SENSITIVE RELEASING SYSTEMS

R. Delatouche[a], M. Mondon[a], F. Collette[d], Ch. Bachmann[b], G. Frapper[b], Ch. Len[c], V. Heroguez[d], Ph. Bertrand[a].

[a] Laboratoire Synthèse et Réactivité des Substances Naturelles, UMR CNRS 6514, 40 Avenue du Recteur Pineau, 86022 Poitiers Cedex, France
[b] Laboratoire Catalyse en Chimie Organique, UMR CNRS 6503, Université de Poitiers, 40 Avenue du Recteur Pineau, 86022 Poitiers Cedex, France
[c] UTC/ESCOM, TIMR EA 4297, Centre de Recherche Royallieu, BP 20529, F-60205 Compiègne Cedex, France
[d] Laboratoire de Chimie des Polymères Organique, UMR CNRS 5629, 16 Avenue Pey Berland, 33607, Pessac cedex, France

Recent achievements in the nanoparticles-based, especially polymer-based nanoparticles targeted therapies, are due to the exploitation of particular biological processes found in cells and to the discovery of physiological differentces between normal and abnormal tissue environments. In the specialized context of cancers, these achievements allowed the approval of some therapeutic solutions where the tumour permeability and the cell endocytosis are exploited (Figure 1).

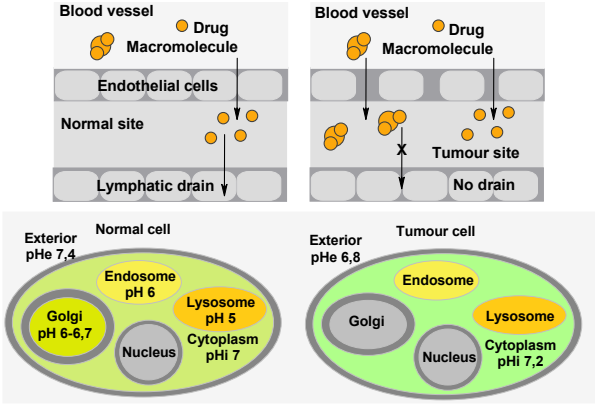


Fig. 1. EPR effect and cellular pHs.

Meada proposed three decades ago to use the physiological differences found between normal and tumours tissues [1]. In a normal tissue, the vascular system is well organized and with a permeability allowing small molecules to pass through, while in tumour tissues, the vascular system is generated by the tumour itself and is poorly organized, with a final higher permeability. The lymphatic drain is not working properly also. Thus elements reaching the tumour site are accumulated and never escape. This concept was called enhanced permeability and retention effect (EPR). The idea proposed was to use

the particles with convenient sizes, so that they can easily circulate in the blood vessels until they reach the tumour tissues. Being big enough to avoid the crossing of the endothelial cells in normal tissues, they should be small enough to pass through the more permeable tumour vascular system. The generally accepted size ranges from 10 to 200 nanometers. Below this size, the renal excretion can be involved while macrophages can trap the objects of bigger sizes. This targeting strategy is called „passive targeting" and theoretically does not require additional properties. In order to be sure that the particles are not interacting with any elements during blood circulation, especially macrophages, pegylation has been widely used to coat these particles [2, 3]. PEG chains are positioned at the outer surface of the particles, masking its content from the exterior, allowing higher hydrophobicity and giving stealth properties. These external PEG chains can also be modified by several means to modify the final behaviour of the particles.

Independently of the passive targeting, the active targeting is based on markers over expressed in the environment of tumour cells [4]. Prodrugs can be designed where the therapeutic agent is embedded in a construct able to reached the tumour environment, and then specifically interact with tumour cells to release the therapeutic agent. This can be made out of the cell, and the therapeutic agent must then diffuse in cells through their membranes, a limitation that can lead to possible diffusion out of the tumour zone and produce side effects. To avoid these potential problems more recent work is devoted to systems designed to release the therapeutic agent in cells only, a strategy based on the acidic tumoral environment [5]. To achieve this aim, the natural cell endocytosis was exploited by several groups. During endocytosis, objects are internalized by cells for their needs. This process starts with the invagination of the membrane (Figure 2), leading to an intramolecular vesicle called endosomes, with low acidic pH (6). On maturation, these endosomes change to lysosomes with more acidic pH (5) [6]. This process was used by several groups to prepare acidic sensitive prodrugs and the vectors can also be designed to be biodegraded under low acidity [7–9].

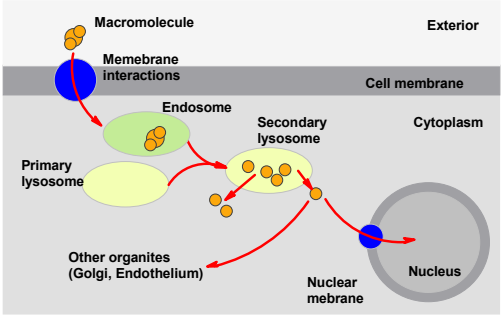


Fig. 2. Simplified mechanism of endocytosis.

Finally, active targeting based on acidic sensitive prodrugs and EPR effects was combined in more efficient therapeutic solutions. Typical applications with polymeric vectors as drug delivery systems and release in cells are shown in Figure 3. Figure 3A illustrates the use of copolymers, where the chain A is not functionalized, the chain B is carrying an additional group used to enhance internalization, chain C bears the active compound in a releasable form (DOX)

and chain D is grafted with a fluorescent dye for cellular imaging. Figure 3B shows conventional delivery systems with HPMA (hydroxypropylmethacryl-amide) [10–13], PEG (polyethyleneglycol) only or PGA (polyglutamic acid) with canonical anti cancer agents.

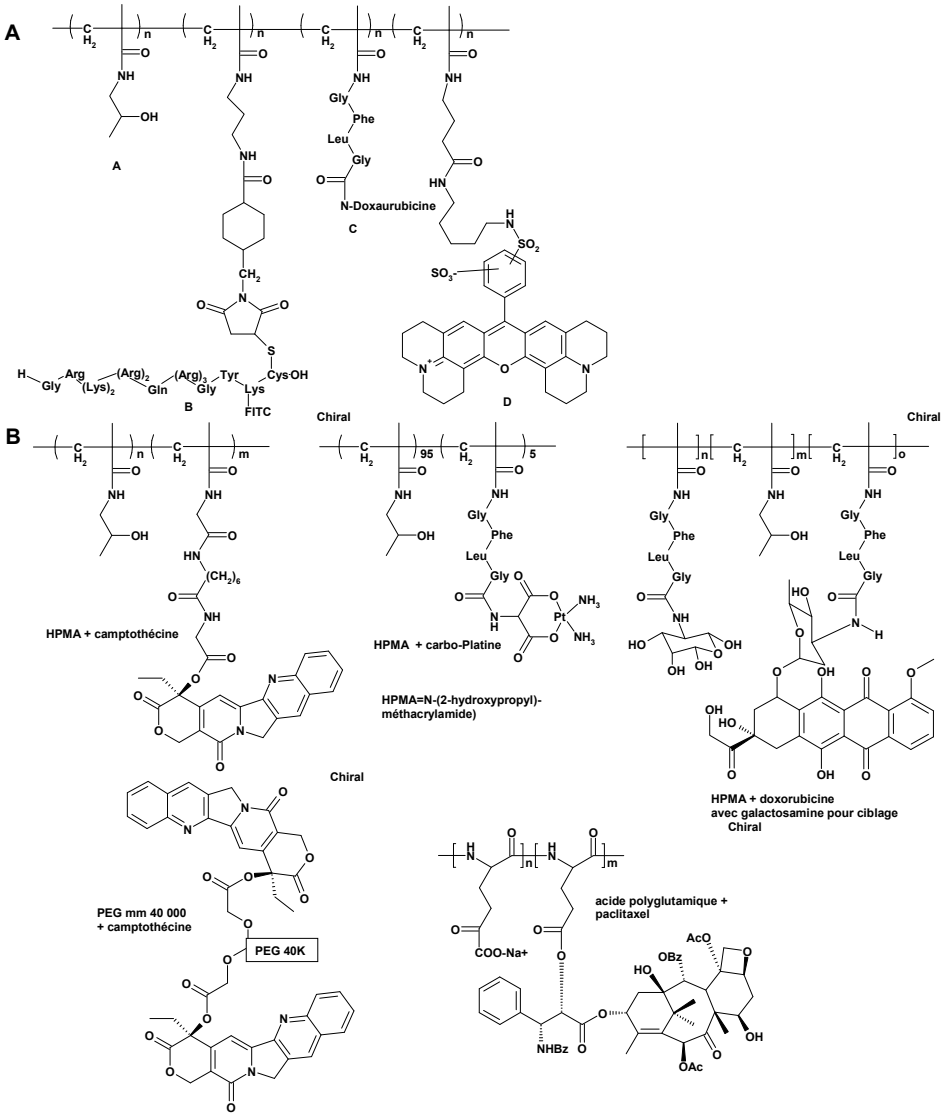
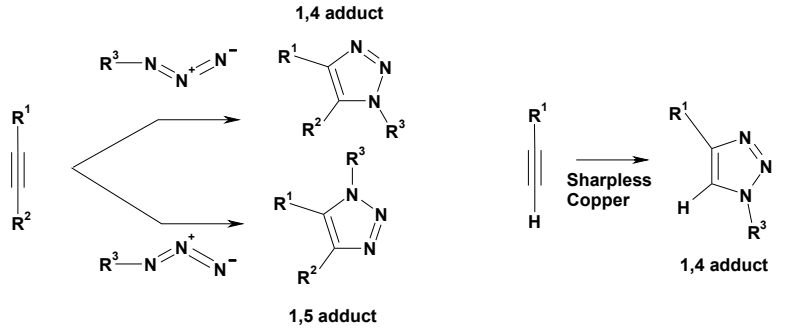


Fig. 3. Examples of polymer-based drug delivery systems.

Our group is involved since several decades in the design of prodrugs for the active targeting and recently we started to work on passive targeting in order to exploit acidic cell endocytosis as releasing mechanism. The functionalization of a drug delivery system can be a complex task depending on the number of functionalities the final particles should have. In Fig. 3A, one can see that from the common HPMA backbone, all four chains are functionalized by different reactions. During recent years, the concept of click chemistry has been introduced by Sharpless [14]. Click chemistry is now a popular term used for simple reactions allowing the assembly of two different chemical elements with high yield. Several reactions can be classified as click reactions: alkyne–azide

cycloaddition [15, 16] to triazoles, S-ene addition to alkylthio derivatives and others. For its simplicity the alkyne-azide cycloaddition has been the most widely used method, and it is now a common tool for particle functionalization. This reaction can give two adducts 1,4 and 1,5 in classical thermal conditions, but when terminal alkynes are used under copper catalysis only the 1,4 adduct is obtained (Scheme 1). Copper free click reactions are also possible in biological media.



Scheme 1. Copper assisted azide–alkyne click reaction (CuACC).

We proposed to combine the preparation of acid-sensitive prodrugs and particle functionalization in a single reaction mediated by the click chemistry. In this concept, conveniently chosen propargylated derivatives of a therapeutic agent could be grafted onto an azido particle to afford directly a particle functionalized with an acid-sensitive system. Several acid-sensitive groups were previously used in such drug delivery strategies, like hydrazone [17], rityles [18], or acetals [19] (Fig. 4), and in fact this releasing strategy can be viewed as a particular application of the chemistry of protecting groups, cleaved under acidic conditions.

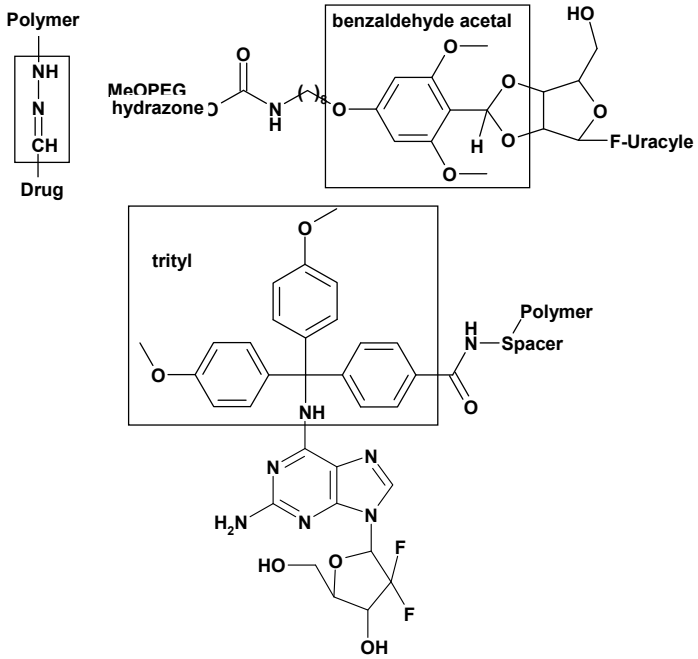
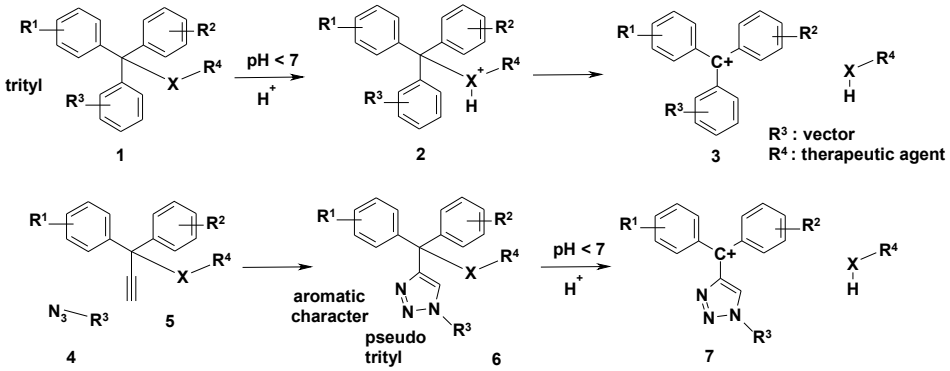


Fig. 4. Acidic releasing systems.

The rational for selecting alkyne–azide click chemistry in our concept was due to the aromatic character of the triazole ring obtained after the cycloaddition, used to replace one of the aromatic rings of the acid-sensitive trityl group (Figure 4 and Scheme 2). The driving force in the releasing mechanism for acid-sensitive groups is the possibility to stabilize the carbocation **3** (Scheme 2) formed after protonation of the prodrug **1**. It was considered that the protonation site is on the atom linking the active molecule to the aci-sensitive group as shown in the protonated form **2**. Thus preparing 2,2-dialkyl- or 2,2-diarylpropargylic derivatives like **5** should give after cycloaddition with an azide **4** the expected dialkyl- or diaryltriazolylmethyl acidic sensitive group **6** as an analogue for the trityl group, the triazole ring being involved in the stabilization of the carbocation **7**.



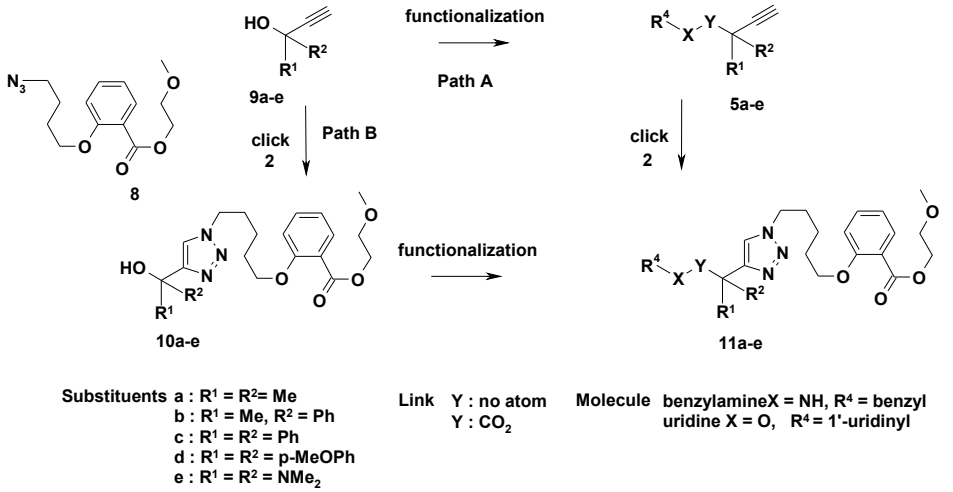
Scheme 2. Concept of direct access to vector functionalized with acid-sensitive systems.

This concept was evaluated on the model compounds, with model for the polymeric vector and models for the therapeutic agents. Alcohols and an amine were selected as representative functional groups find in many therapeutic compounds. In order to extend the scope of this work, carbamate versions for the amine and carbonate versions of the alcohols were also investigated (Scheme 2, X = OC=ONH– or OC=OO–), allowing the discovery of a new releasing mechanism. Synthetic models were tested to determine the hydrolysis rate at various buffered pHs via ^{1}H NMR or HPLC techniques.

The polymer model consisted of a salicylate derivative **8** bearing a short methoxyethyl ester group and azidobutyloxy group (Scheme 3). This model was supposed to mimic the possible use of polyethyleneoxide vectors. Several alkynols **9** were prepared as intermediates towards the acid-sensitive prodrugs **11**. Two paths were investigated (Scheme 3), path A considering the preparation of functional alkynes **5** and subsequent cycloaddition, and path B involving first cycloaddition to give the alcohol **10** and final functionalization.

Several carbamates and alkylamines were prepared [21, 22] from benzylamine and some ethers from a simple alcohol and uridine [22], carbonate found being unstable. All these compounds were submitted to acidic hydrolysis at various pH (Table 1). Acidic (pH = 1), mildly acidic pH (pH = 4.3) and physiological pH (pH = 7.3) were selected. Stability at physiological pH is important considering that the future objects have to circulate in blood vessels before reaching the targeted area. All the tested compounds showed stability at physiological pHs, except one, an amine derivative bearing two anisyl groups

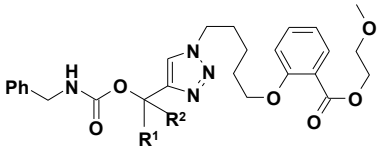
(R^1 = R^2 = An). This instability was attributed to possible direct nucleophilic substitution by water, this type of dianisyl compound being more sensible to such reaction.



Scheme 3. Synthetic strategy.

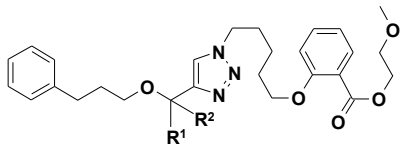
Table 1. **Half-lives ($t_{1/2}$) of carbamates, alkylamines and ethers at various pHs**

Carbamate derivatives



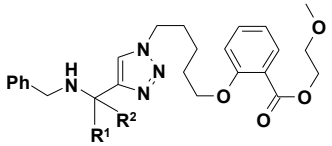
R^1	R^2	$t_{1/2}$, min	
		pH 4.3	pH 7.3
Me	Me	660	stable
Ph	Ph	4.92	stable

Ether derivatives



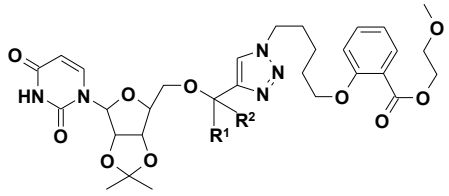
R^1	R^2	$t_{1/2}$, min		
		pH 1	pH 4.3	pH 7.3
Ph	Ph	a	∞b	n. d.
An	An	<20	360-420	n.d

Amine derivatives



R^1	R^2	$t_{1/2}$, min	
		pH 4.3	pH 7.3
Me	Me	stable	stable
Ph	Ph	9000	stable
An	An	1.31	360

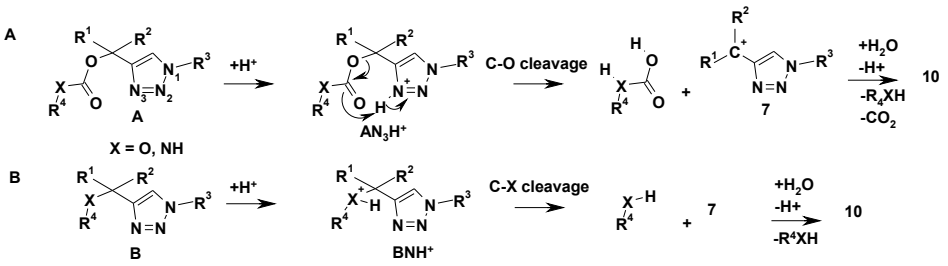
Uridine derivatives



R^1	R^2	$t_{1/2}$, min		
		pH 1	pH 4.3	pH 7.3
Ph	Ph	180	>3600	stable
An	An	<20	3600	stable

These results were then rationalized by molecular modelling, using the B3LYP method. The first step was to determine the protonation sites. Triazoles

alone were known to be protonated mainly on N(3) (Scheme 4). In the case of the carbamate derivatives (Scheme 4A, X = NH), the protonation on the nitrogen atom or the carbonyl group of the carbamate is disfavoured compared to the nitrogen atom N(3) on the triazole ring. After protonation on the N(3) nitrogen atom, a proton shift to the carbonyl group can be observed during computational work. In the case of the carbamate, this participation of the triazole in the hydrolysis is the driving force, leading to the formation of carbamic acid and the carbo cation **7**. The carbamic acid is then decomposed to the corresponding amine and CO_2. This can also be applied theoretically to carbonates, although we were unable to synthesize them to confirm the calculations. In the case of aniline and all ethers derivatives, the mechanism involves the respective protonation of the nitrogen and oxygen atoms (Scheme 4B, X=O or NH), with rapid subsequent C–N or C–O bond break to give the carbo cation **7**. In all cases, carbocation **7** is trapped by the surrounding water to give the alcohol **10**. This modelling work also demonstrated that the more the carbocation is stabilized, the faster the hydrolysis is.



Scheme 4. General hydrolysis mechanisms.

In conclusion, our initial concept corresponding to path A appeared to be working in some cases, when the propargylic derivatives **5** are by themselves stable enough to undergo cycloaddition without side reactions. Path A was also applied to aniline (unpublished work, instead of benzylamine) and the cycloaddition was observed to proceed in modest yields with the major reaction being an intramolecular cyclization to oxazolidinones. Path B appeared to be synthetically more practical and was adapted to develop this work in the context of particles functionalized with acid-sensitive systems for the release of bioactive molecules.

ACKNOWLEDGEMENTS

Authors thank CNRS and ANR for financial support and ANR for RD grant (ANR-08-PCVI-030).

R E F E R E N C ES

1. Maeda, H. (20100. *Bioconjugate Chem.*, *21*, 797.
2. Kabanov, A.V., Batrakova, E.V., Alakhov, V.Y. (2002). *Adv. Drug. Dev. Reviews*, *54*, 759–779.
3. Ceh, B., Winterhalter, M., Frderik, P.M., Vallner, J.J., Lasic, D.D. (1997). *Adv. Drug Dev. Reviews*, *24*, 165.
4. Wu, A.M., Senter, P.D. (2005). *Nature Biotechnol.*, *23*, 1137.
5. Prescott, D.M., Charles, H.C., Poulson, J.M., Page, R.L., Thrall, D.E., Vujaskovic, Z., Dewhirst, M.W. (2000). *Clin. Cancer Res.*, *6*, 2501.
6. White, S., Szewczyk, J.W., Turner, J.M., Baird, E.E., Dervan, P.B. (1998). *Nature*, *391*, 468.
7. Guo, X., Szoka, Jr., F. C. (2001). *Bioconj. Chem.*, *12*, 291.

8. Asokan, A., Cho, M.J. (2004). *Bioconj. Chem.*, *15*, 1166.
9. Heffernan, M.J., Murthy, N. (2005). *Bioconj. Chem.*, *16*, 1340.
10. Bachelder, E.M., Beaudette, T.T., Broaders, K.E., Paramonov, S.E., Dashe, J., Fréchet, J.M. (2008). *J. Mol. Pharm.*, *5*, 876.
11. Ulbrich, K., Subr, V. (2010). *Adv. Drug Deliv. Rev.*, *62*, 150.
12. Duncan, R., Gac-Breton, S., Keane, R., Musila, R., Sat, Y.N., Satchi, R., Searle, F. *J. Contr. Release*, *74*, 135.
13. Kopecek, J., Nori, A. (2005). *Adv. Drug Deliv Rev.*, *57*, 609.
14. Kolb, H.C., Finn, M.G., Sharpless, K.B. (2001). *Angew. Chem., Int. Ed.*, *40*, 2004.
15. Huisgen, R. (1963). *Angew. Chem., Int. Ed.*, *2*, 565.
16. Huisgen, R. (1963). *Angew. Chem., Int. Ed.*, *2*, 633.
17. Etrych, S., Sirova, M., Starovoytova, L., Rihova, B., Ulbrich, K. (2010). *Mol. Pharm.*, Articles ASAP, DOI: 10.1021/mp100119f
18. Patel, V.F., Hardin, J.N., Mastro, J.M., Law, K.L., Zimmermann, J.L., Ehlhardt, W.J., Woodland, J.M., Starling, J.J. (1996). *Bioconj. Chem.*, *7*, 497.
19. Fréchet, J. M. J., Gillies, E. R., Goodwin, A. P. *Bioconjugate Chem.* **2004**, *15*, 1254.
20. Bertrand, P., Gesson, J.-P. (2007). *J. Org. Chem.*, *72*, 3596
21. Delatouche, R., Mondon, M., Gil, A., Frapper, G., Bachmann, C., Bertrand, P. (2011). Novel Triazolyl Derivatives for Acidic Release of Amines. *Tetrahedron*, *67*, 401.
22. Mondon, M., Delatouche, R., Bachmann, C., Frapper, G., Len, C., Bertrand, P. (2011). *Eur. J. Org. Chem.*, 2111.
23. Delatouche, R., Lesage, A., Collette, F., Héroguez, V., Bertrand, P. (2011). *Austral. J. Chem.*, *64*, 166.