Journal of the Mexican Chemical Society
ISSN: 1870-249X
editor.jmcs@gmail.com
Sociedad Química de México
México

Madrigal, Domingo; Pina Luis, Georgina; Rivero, Ignacio A.

Synthesis and Hydrolysis Monitoring of Sasrin-like Resin Bound Imines by Fluorescence Spectroscopy

Journal of the Mexican Chemical Society, vol. 50, núm. 4, 2006, pp. 175-179

Sociedad Química de México

Distrito Federal, México

Article

J. Mex. Chem. Soc. **2006**, *50(4)*, 175-179

ISSN 1870-249X

Synthesis and Hydrolysis Monitoring of Sasrin-like Resin Bound Imines by Fluorescence Spectroscopy

Domingo Madrigal, Georgina Pina-Luis, Ignacio A. Rivero*

Centro de Graduados e Investigación. Instituto Tecnológico de Tijuana. Apartado Postal 1166. 22000. Tijuana, B. C. México.
E-mail: irivero@tectijuana.mx

Recibido el 4 de abril de 2006; aceptado el 8 de diciembre del 2006.

Abstract. Merrifield resin was converted to various dialkyl-trihydroxybenzaldehyde resins and then to different bound imine resins. Fluorescence spectroscopy was used to continuously monitor the intermediates performed on the resins. Using fluorescent label (dansyl chloride) information on the kinetics of the amine cleavage was obtained. This study demonstrated that direct measures of fluorescence intensity on single-resin beads can be an effective method of monitoring solid phase organic reactions.
Key words: Fluorescence spectroscopy, monitoring reaction, solid phase.

Resumen. Se empleó resina de Merrifield para obtener varias resinas con grupos de trihidroxibenzaldehido dialquilados y posteriormente se obtuvieron las correspondientes iminas. Los intermediarios formados sobre las resinas fueron monitoreados usando espectroscopia de fluorescencia. Se obtuvo la cinética de liberación de la amina usando un indicador fluorescente (cloruro de dansilo). Este estudio sugiere que la intensidad de fluorescencia obtenida por medida directa desde una muestra de resina puede ser un método eficiente para monitorear reacciones en fase sólida.
Palabras clave: Espectroscopía de fluorescencia, monitoreo de reacciones, fase sólida.

Introduction

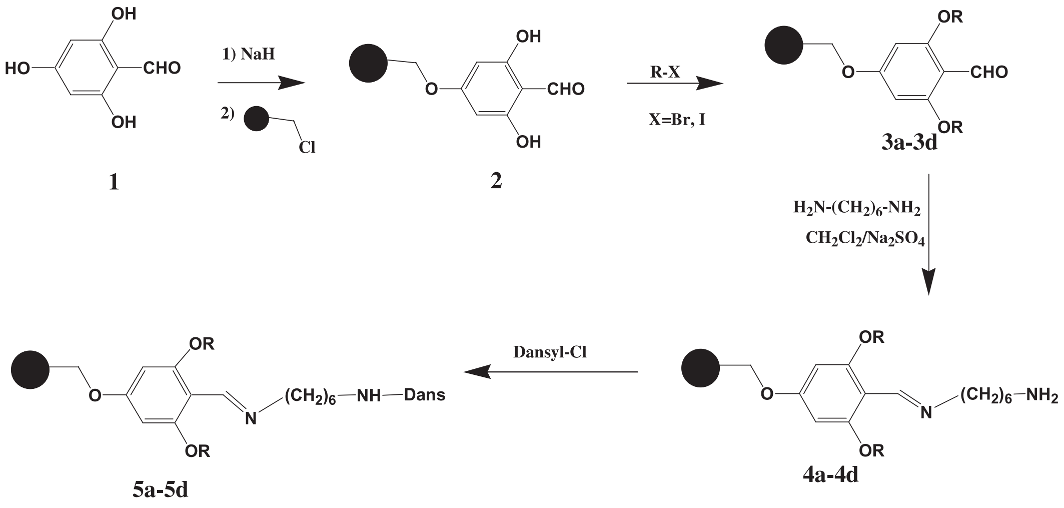
An important asset during the development of new solid phase organic synthesis (SPOS) is not only the possibility of rapidly determine whether a synthetic step has proceeded to completion, but also in getting a deeper understanding in reaction kinetics on solid phase. Among the different analytical methods [1-5] available for detection on SPOS those relying on fluorescence [6,7] detection have not been widely exploited [8]. Fluorescence spectroscopy offers distinct advantages in terms of sensitivity, selectivity, response time, local observation (e.g. by fluorescence imaging spectroscopy), time discrimination (e.g. by implementation of time-gating procedures). Moreover, *in situ* continuous monitoring is possible using optical fibers.

Benzaldehyde resin is known to be useful in solution phase synthesis for removing excess nucleophilic reagents and to scavenge primary amines [9,10]. In addition, it is useful for preparing amine libraries by solid phase methods due to the fact that products can be easily cleaved from the resin by mild acid hydrolysis [11,12]. Based on our previous works in which reactions carried out on different solid supports were followed by fluorescence spectroscopy [13-17], in this paper we report on a model study, conditions that allow the direct fluorimetric monitoring of the release of amines from resins. We started our study with the modification of Merrifield resin into a SASRIN-type dihydroxybenzaldehyde resin and then to representative set of hydroxy-protected alkyl resins. Finally, hexamethylenediamine was coupled to afford different bound imine resins. Since the starting Merrifield resin displayed native fluorescence (due to the intrinsic styrene-divinylbenzene backbone fluorophore), we deduced that it could be used as an internal fluorescent probe to monitor ongoing chemical reactions performed on the resin. As we demonstrate, the range of reactions that can be monitored by this on-resin method were confined to those in which no-spectral overlap existed among the species of interest or to those in which the chemical transformation altered the π-electronic system of the fluorophore. It was found that acid hydrolysis of the immobilized amines did not fulfill the above requirements and conversion of the attached amines to the corresponding dansyl derivative was necessary. Cleavage of the fluorescent labeled amines can be used to obtain information on the kinetics of the hydrolysis reaction on the different substituted resins.

Results and Discussion

The general solid phase route, which is shown in Scheme 1, began by attachment of 2,4,6-trihydroxybenzaldehyde **1** to Merrifield resin to deliver the 4-formyl-3,5-dihydroxyphenoxy-PS resin **2**. The generation of the immobilized aldehyde species **2** could be conveniently monitored by fluorescence spectroscopy without any manipulation of the resin except to transfer it to a conventional flow-through fluorescence cell.

As illustrated in Figure 1, under the experimental conditions, the SASRIN-like resin **2** excitation and fluorescence spectrum are shifted (ca. 250 nm) compared to that of Merrifield resin. These red shifts could be ascribed to the presence of carbonyl groups in PS-4-formyl-3,5-dihydroxyphenoxy **(2)** resins. The fluorescence spectra of most carbonyl-containing compounds are characterized by the presence of an n - π* band of low intensity corresponding to the electronic transition in the carbonyl group and appear at longer wavelengths. Consequently spectra of derivatives **2**, show a fluores-



Resin	R
a	Me
b	Et
c	*i*-Pr
d	Benzyl

Scheme 1. Synthetic route of Resin-imine

cence band of smaller intensity at 650nm. Transformation of Merrifield resin to the resin **2** one was deemed to occur quantitatively on account of the complete disappearance of the 390 nm fluorescence band of the Merrifield resin and the emergence of the new fluorescence band at 650 nm. Based on the assumption of a quantitative conversion, the validation of the approach was assessed by chlorine analysis [18]. Starting with 200 mg of Merrifield resin (loading 1.19 mmol/g) the Volhard titration gave a chlorine content of cero, indicating a conversion of 100% [19]. Additionally, mass spectrometry direct insertion of resin **2**, showed the ion molecular ([M$^+$] 154) and typical fragmentation patterns of 2,4,6-trihydroxybenzaldehyde [20]. The dansyl derivatives **5a-5d** showed peaks with *m*/*z* 316 as result of typical McLafferty elimination from α-carbon of imine nitrogen, one peak of dansyl fragment with mass of 235 and dimethylaminonaphtalene peak with *m*/*z* of 170.

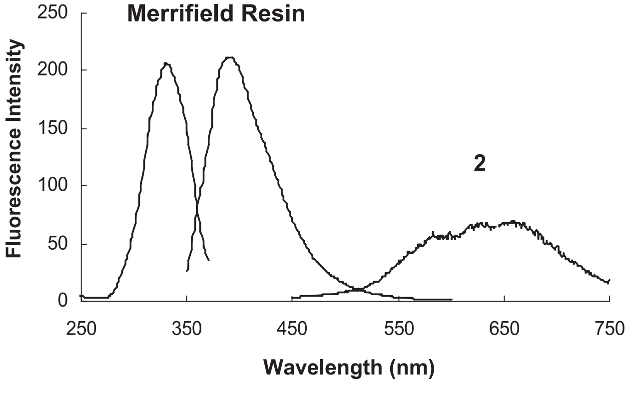


Fig. 1. Fluorescence spectra of Merrifield and PS-4-formyl-3,5-dihydroxyphenoxy (**2**) resins.

According to Scheme 1, the resin **2** was reacted with methyl iodide in Na_2CO_3/DMF. The same reaction was run employing different alkyl halides in order to obtain a library of hydroxyprotected resins **3a-3d**. Single-resin beads were transferred to the fluorescence flow-cell and the spectra were recorded. As shown in Figure 2, all spectra showed an increase of the fluorescence intensity as well as similar band shape and fluorescence maximum, with a greater fluorescence efficiency of the corresponding resin **3d.** This last result, could be due to the influence of more inductive effect of the two benzyl groups in the π-electron system of the resin. Differentiation among similar resins **3a-3d** was, in this case, hard to achieve by only measuring their native fluorescence spectral characteristics.

Functionalized resins **3a-3d** was reacted with hexamethylenediamine to give supported imine **4a-4d**. Substitution of aldehyde functionality by the imine one resulted in a decrease of fluorescence intensity and in a blue shift on excitation and fluorescence wavelengths. However, as already observed for resins **3a-3d**, the negligible spectral fluorescence differences of the several supported imine **4a-4d** resins did not allow distinguishing effectively among them. These results prompted us

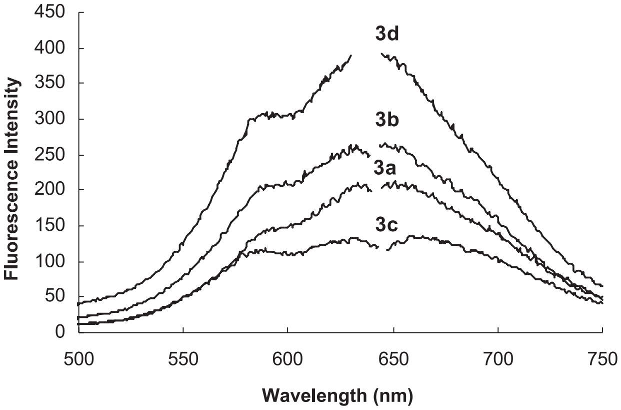


Fig. 2. Fluorescence excitation and emission spectra of alkyl derivatives resin **3a-3d.**

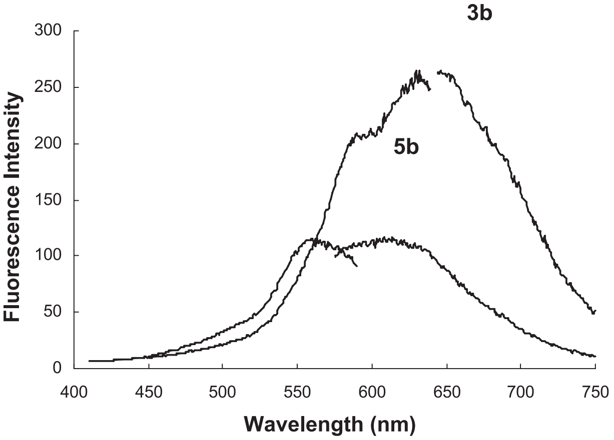


Fig. 3. Fluorescence excitation and emission spectra of dansylated derivative Resin-imine-Dansyl (**5b**) and Resin-dialkyltrihydroxybenzaldehyde (**3b).**

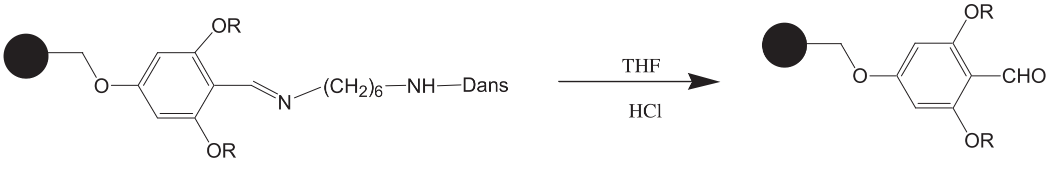
to derivatize the supported imine-amine 4a-4d resins in order to study the kinetics of its cleavage from the different resins and the influence of the protecting groups on the kinetics. So, the supported imine 4a-4d resins were finally reacted with dansyl chloride to obtain the corresponding fluorescent derivatives 5a-5d. The position of the excitation and emission fluorescence maximum of the 5a-5d derivatives (λ_{exc} = 560 nm) (λ_{em} = 610 nm) and the 3a-3d derivatives (λ_{exc} = 585 nm) (λ_{em} = 650 nm) (as can be observed in Figure 3 for 3b and 5b) permitted a much more precise determination of spectral changes whilst simultaneously monitoring the progress of the cleavage reaction (Scheme 2). On the other hand, in order to use a control, incorporation of the dansyl probe onto the raw resin showed a wide emission band around 600 nm. This behavior was in correspondence with previous works [13-17], that reported fluorescence emission bands of solid phase dansyl derivatives in a wide range between 530 to 630 nm. The terminal position of the dansyl group in the amine long chain gives rise to energy losses, may be due to non radiative process which adversely affects the fluorescent intensity. Therefore a remarkable increase in the intensity of fluorescence with the dansyl immobilization was not observed.

The position of the excitation and emission fluorescence maximum of the **5a**-**5d** and **3a**-**3d** each derivatives (as can be seen in Figure 3 for derivatives **3b** and **5b**) permits a precise determination of spectral changes, whilst simultaneously monitoring the progress of the cleavage reaction (Scheme 2). We followed the hydrolysis reaction of the all **5a**-**5d** to **3a**-**3d** derivatives, used a solution of THF-HCl (4:1 v/v). The resins **5a**-**5c** showed similar kinetics in the hydrolysis (reaction times <30 min) while the resin **5d** with benzyl groups exhibited longer kinetics (ca. 120 minutes).

Fluorescence spectra during the conversion of the resin **5b** to **3b** (hydrolysis reaction) at various times were obtained and monitorized by following the disappearance of immobilized dansyl resin band at 610 nm and the emergence of the aldehyde-resin band at 650 nm (Figure 4A). The progression of the fluorescence intensity of the **5b** resin band with respect to the intensity of the **3b** resin peak at 650 nm was plotted against time. In Figure 4B the time course of the hydrolysis reaction of **5b** is shown. Additionally, we analyzed kinetics data based on the rationalizations reported by Yan [7,21] and by us to study the reaction kinetics of hydroxyl resins conversion into the chlorinated ones [13]. A pseudo first-order rate constant of 2.42×10^{-4} sec^{-1} was obtained by fitting with equation: $P = 1 - e^{-kt}$ where (P) is the product in the bimolecular reaction:

$$\text{Bead} + \text{R} \rightarrow \text{P}$$

and the reactant (R) is in large excess. Similar values of the kinetic constant were found for the conversion **5 (a, c, d)** to **3 (a, c, d)** resins.



5a-5d

3a-3d

Scheme 2. Imine release.

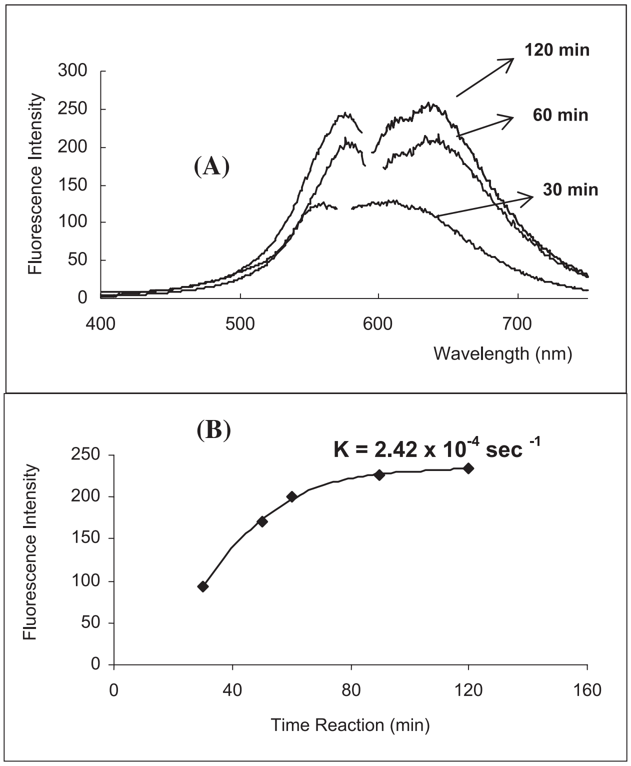


Fig. 4. (**A**) Fluorescence spectra during the transformation of resin **5b** in **3b** at different times. (**B**) Fluorescence intensity of the **3b** resin peak at 650 nm against time.

Conclusions

An easy method to synthesize resins bound imines using Merrifield resin as solid support was made in good yields. The cleavage of amines from the resin by acid hydrolysis is an important step in the preparation of amine libraries by solid phase synthesis. Fluorescence spectroscopy was successfully used to evaluate the different reaction steps and to determine not only if the reaction has proceeded to completion, but also the kinetic of the amine release. This fat indicates that there is a uniform distribution of reactive sites throughout the beads as was proposed by Bradley [22] to PS resins. The results obtained, allow to propose a method to evaluate the influence of the alkyl substitute groups in the rate of imines hydrolysis using fluorescence spectroscopy to derivatives of Merrifield resin. The imine group quenches the signal and the hydrolysis products recuperate to baseline.

Experimental Section

Merrifield (1.19 mmol/g Cl) and other reagents, if not specified were purchased from Aldrich Chem. Co. Infrared (IR) spectra were taken on a Perkin Elmer FT-IR 1600 spectrometer. Mass spectra were obtained on a Hewlett Packard 5989 MS Spectrometer at 70 eV by direct insertion. The Fluorescence spectra were obtained on Spectrofluorometer Shimadzu RF-5301 PC. Beads were washed free of any starting materials and solvents and were placed into a Hellma model 176.52 flow through cell (25 mL) for fluorimetric analysis.

4-formyl-3,5-dihydroxyphenoxy-PS **(2).** In a typical experiment a solution of 2,4,6-trihydroxybenzaldehyde (1.8 g, 11.9 mmol) in DMF (30 mL) was cooled at 0ºC in an ice bath and sodium hydride (0.342 g, 14.25 mmol) was slowly added. The reaction mixture was warmed to room temperature and after gentle stirring for 15 min, Merrifield resin (5 g, 5.95 meq/Cl) was added in one portion. The reaction mixture was warmed in a water bath and was stirred for 24 h. Then, the resin was filtered, washed DMF, MeOH, water, MeOH, CH_2Cl_2, (3 x 20 mL each) and dried under high vacuum for 24 h to yield resin **2**. The yield was 90% of recuperation and 100% conversion. IR (KBr): 3448, 3025, 2912, 1653, 1600 cm^{-1}. MS (*m/z*): 154 (100%) $[M^+]$, 153(94%) $[M^+-1]$, 108(11%).

General method of alkylation on resin

4-formyl-3,5-dialkyloxyphenoxy-PS **(3a-3d):** To 10-mL reactor tubes, resin **2** was added (500 mg, 0.595 meq/Cl), followed by the addition of DMF (9 mL) and Na_2CO_3 (126 mg). The Resin-THB **2** was alkylated with methyl iodide (337 mg, 2.38 mmol), ethyl bromide (259 mg, 2.38 mmol), *iso*-propyl bromide (292 mg, 2.38 mmol) and benzyl bromide (406 mg, 2.38 mmol). The reaction mixtures were stirred at 72 h at 80ºC. Finally, resins were washed with EtOH, water, EtOH and $CHCl_3$, (3 x 10 mL each), and dried at high vacuum for 24 h.
3a: 0.47 g. IR (KBr): 3449, 3422, 1651, 1619 cm^{-1}. EF: λ_{ex} = 634, λ_{em} = 657, I_f = 205. **3b:** 0.48 g. IR (KBr): 3449, 3412, 1646, 1623 cm^{-1}. EF: λ_{ex} = 632, λ_{em} = 654, I_f = 258. **3c:** 0.46 g. IR (KBr): 3423, 3415, 1651, 1619 cm^{-1}. EF: λ_{ex} = 629, λ_{em} = 666, I_f = 128. **3d:** 0.49 g. IR (KBr): 3449, 3422, 1651, 1619 cm^{-1}. EF: λ_{ex} = 630, λ_{em} = 652, I_f = 377.

General method to synthesis of resin-imine.

Resin-Imine (**4a-4d**): The resins **3a-3d**, were subsequently treated with CH_2Cl_2 (9 mL), hexamethylenediamine (103 mg, 0.89 mol) and Na_2SO_4 anhydrous (710 mg, 5 mmol). After stirring the reaction mixtures for 24 h at 50ºC, the solvent was drained and the resins washed with EtOH, water, EtOH, and CH_2Cl_2 (3 x 10 mL each), and dried under high vacuum for 24 h to yield resins **4a-4d.**

4a: 0.47 g. IR (KBr): 3449, 3422, 1651, 1619 cm^{-1}. EF: λ_{ex} = 582, λ_{em} = 637, I_f = 101. **4b:** 0.48 g. IR (KBr): 3449, 3412, 1646, 1623 cm^{-1}. EF: λ_{ex} = 584, λ_{em} = 643, I_f = 64. **4c:** 0.46 g. IR (KBr): 3423, 3410, 1651, 1619 cm^{-1}. EF: λ_{ex} = 578, λ_{em} = 638, I_f = 90. **4d:** 0.49 g. IR (KBr): 3449, 3422, 1651, 1619 cm^{-1}. EF: λ_{ex} = 542, λ_{em} = 596, I_f = 228.

General method to synthesis of resin-imine-dansyl

Resin-Imine-Dansyl (**5a-5d**): The resin beads **4a-4d** (455 mg) were suspended in CH_2Cl_2 (10 mL), followed by addition of dansyl chloride (174 mg, 0.65 mmol) and Na_2CO_3 (69 mg, 0.65 mmol). The reaction mixtures were kept at room temperature for 2 h in an ultrasonic bath, after which the fluorescent labeled materials **5a-5d** were filtered and washed with EtOH, water, EtOH and CH_2Cl_2, (3×10 mL each), and dried under high vacuum for 24 h to yield resins **5a-5d.**

5a: 0.40g. IR (KBr): 3449, 1651, 1600, 1568, 1440 cm^{-1}. EF: $\lambda_{ex} = 558$, $\lambda_{em} = 610$, $I_f = 114$. MS *m/z* (%): 316 (5), 235 (2), 203 (12), 171 (100). **5b:** 0.43 g. IR (KBr): 3448, 1651, 1598, 1490, 1440 cm^{-1}. EF: $\lambda_{ex} = 562$, $\lambda_{em} = 616$, $I_f = 66$. MS *m/z* (%): 316 (10), 235 (9), 203 (33), 171 (100). **5c:** 0.42 g. IR (KBr): 3449, 1651, 1600, 1573, 1435 cm^{-1}. EF: $\lambda_{ex} = 563$, $\lambda_{em} = 629$, $I_f = 119$. MS *m/z* (%): 316 (11), 235 (3), 203 (29), 171 (100). **5d:** 0.45 g. IR (KBr): 3449, 1651, 1600, 1577, 1440 cm^{-1}. EF: $\lambda_{ex} = 552$, $\lambda_{em} = 607$, $I_f = 214$. MS *m/z* (%): 316 (10), 235 (9), 203 (31), 171 (100).

Method of imine hydrolysis

Resin **5b** (109 mg) was treated with a solution of THF-HCl (4:1 v/v) (10 mL). Reaction mixture was stirred at room temperature. A sample aliquot (1mL) was taken every 30 min. The resin was washed with water, methanol, and CH_2Cl_2 (3x10mL each) and dried at high vacuum for 24 h to yield resin **3b** (Figure 4A, B).

Acknowledgments

We gratefully acknowledge support for this project by Consejo Nacional de Ciencia y Tecnología, México (CONACyT, GRANT No. SEP-2004-C01-47835) and Consejo Nacional de Educación Tecnológica, México (COSNET, GRANT No. 23.97-P). Thanks to Dr. Daniel Chávez for mass spectrometry data.

References

1. Yan, B.; Gstach, H. *Tetrahedron Lett.* **1996**, *37*, 8325-8328
2. Gosselin, F.; Di Renzo, M.; Ellis, T.H.; Lubell, W.D. *J. Org. Chem.* **1996**, *61*, 7980-7981
3. Shapiro, M.J.; Gounarides, J.S. *Prog. Nucl. Magn. Reson. Spect.* **1999**, *35*, 153-200
4. Stöver, H.D.; Fréchet, J.M. *Macromolecules*, **1989**, *22*, 1574-1576
5. Taylor, E.V.; Qian, M.G.; Dollinger, G.D. *Anal. Chem.* **1998**, *70*, 3339-3347
6. Yan, B.; Li, W. *J. Org. Chem.* **1997**, *62*, 9354-9357
7. Yan, B.; Martin, P.C.; Lee, J. *J. Comb. Chem.* **1999**, *1*, 78-81
8. Chu, S. S.; Reich, S. H. *Bioorg. Med. Lett.* **1995**, *5*, 1053. Shapiro, M. J.: Kumaravel, G.; Setter, R. C.; Beveridge, R. *Tetrahedron Lett.* **1996**, *37*, 4671-4674
9. Kaldor, S.W.; Seigel, M.G.; Fritz, J.E.; Dressman, B.A.; Hahn, P.J. *Tetrahedron Lett.* **1996**, *37*, 7193-7196
10. Frechet, J.M.; Schuerch, C. *J. Am. Chem. Soc.* **1971**, *93*, 492-496
11. Mergler, M.; Taner, R; Gosteli, J.; Grogg, P. *Tetrahedron Lett.* **1988**, *29*, 4005-4008. Larsson, E.; Lüning, B. *Tetrahedron Lett.* **1994**, *35*, 2737-2738
12. Guiles, J. W.; Johnson, S. G.; Murria, W. V. *J. Org. Chem.* **1996**, *61*, 5169-5171
13. Pina-Luis, G.; Badía, R.; Díaz-García, M.E.; Rivero, I.A. *J. Comb. Chem.* **2004**, *6*, 391-397
14. Rivero, I. A.; González, T.; Pina-Luis, G. *J. Comb. Chem.* **2005**, *6*, 46-53
15. Rivero, I. A.; Peralta, M.; Heredia. S.; Madrigal, D.; Pina-Luis, G. *Arkivoc*, **2003**, *(xi)*, 27-36
16. Castillo, M.; Pina-Luis, G.; Diaz-Garcia, M. E.; Rivero, I. A. *J. Braz. Chem. Soc.* **2005**, *16 (3A)*, 412-417
17. Rivero I.A.; González T.; Díaz-García M.E. *Comb. Chem.& High Throughput Screening*, **2006**, *9(7)*, 535-544
18. Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, **1969,** W. H. Freeman, San Francisco
19. Reaction of **1** with the model compound benzylchloride instead of the Merrifield resin, in THF resulted in a 8:2 *para*:*ortho* ratio mixture of substituted benzyl, which was characterized as follows**.** ^{1}H-NMR (DMSO, 200 MHz): d 10.02 (20%) and 10.09 (80 %) ppm, CHO. Laibinis [17], found an 9:1 ratio of *para*- to *ortho*-substituted products.
20. Chávez, D.; Ochoa, A.; Madrigal, D.; Castillo, M.; Espinoza, K.; González, T.; Vélez, E.; Meléndez, J.; García, J. D.; Rivero, I. A. *J. Comb. Chem***.** **2003**, *5*, 149-154
21. Yan B.; Fell J. B.; Kumaravel, G. *J. Org. Chem.* **1996,** *61*, 7467-7472.
22. Kress, J.; Zanaletti, R.; Rose, A; Frey, G. J.; Brocklesby, W. S.; Ladlow, M; Bradley, M. *J. Comb. Chem.* **2003**, *5*, 28-32.