Combinatorial Chemistry & High Throughput Screening, **2007**, *10*, 261-267

Development of Self-Indicating Resin

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Abstract: Previously, we have reported the development and application of self-indicating resins (SIR), materials which can indicate presence or absence of amines in the reaction solution by the conspicuous color change of a phenolsulfophthalein type dye immobilized on resin beads [2a]. Although the functionality necessary for attaching the dye to the resins could be readily introduced by the Suzuki-Miyaura coupling during the synthesis of the SIR **1**, this approach was only applicable to the dyes containing suitable functionality for the cross-coupling reaction. In this article we describe a new approach of immobilizing the indicating dyes onto the resin support. The dyes suitable for loading onto aminomethyl polystyrene (PS) resin were prepared by Friedel-Crafts reaction of 2-sulfoterephthalic anhydride with a wide range of phenols. Using this new route, the SIR **6c** was readily prepared in >100g quantities. Use of the SIR **6c** in the synthesis of a 144 member urea library was demonstrated and the SIR successfully indicated the endpoint of the reaction between amines and isocyanates.

INTRODUCTION

In recent years, an extensive range of functionalized polymers has been developed for the use in organic synthesis. A variety of functional components such as catalysts, reagents and scavengers have been immobilized onto polymer supports which offer a clean and simple route to compound isolation without the need for product purification [1]. In this regard, we are particularly interested in the immobilization of chemical probes onto traditional Merrifield types of solid phase synthesis resins. Previously, we reported the development of self-indicating resins (SIRs) such as **1** (Fig. **1**), which could be used as a sensor for *in-situ* reaction monitoring [2]. These SIRs were prepared by immobilization of the carboxy modified dye onto an aminomethyl polystyrene resin with the carboxylic acid group introduced *via* a Suzuki-Miyaura cross-coupling reaction of bromophenol blue with 4-carboxylphenylboronic acid. The presence or absence of amines could then be detected by the conspicuous color change of the immobilized bromophenol blue (BPB) derivative.

This modified bromophenol blue dye could also be attached to isocyanate resins using a 1,5-diaminopentane spacer, with the isocyanate resin not only scavenging amine from solution but also indicating the presence of residual amine left in solution. Utility of the scavenger resin was demonstrated during the solution-phase synthesis of a small library of ureas [2b]. More recently, we also developed pH indicating resins through the covalent attachment to solid phase resin of both sulfophthalein dyes and methyl red in order to extend the pH range [2c]. In this article, we report the development of new types of SIRs involving a new approach to dye-immobilization that allows tuning of the transition interval of the dye. The new route is also more suitable for larger scale production of SIRs.

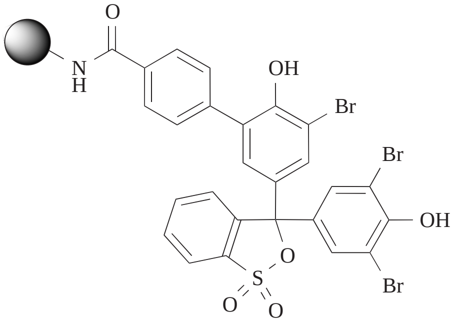

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Fig. (1).

RESULTS AND DISCUSSION

The new synthetic routes to the SIR **6** are depicted in Scheme **1**. Synthesis of **6** starts from terephthalic acid **2**, with sulfonation using fuming sulphuric acid and iron chloride as the catalyst to give the sulfonic acid derivative **3** (57%) [3]. The sulfonic acid **3** was then treated with acetic anhydride to give the anhydride **4** (64-71%) [4]. The Friedel-Crafts reaction of **4** with phenols was carried out by heating the mixture of **4** with the phenols at 140 ~ 180ºC in the presence of $ZnCl_2$ catalyst with or without solvent [4].

The transition interval (TI) of the resulting dye depends on the pKa of the phenol used. Use of a phenol with electron donating substituents (higher pKa) shifted the TI of the dye to the higher pH range while electron withdrawing groups (lower pKa) to the lower pH range. The usual component of BPB, 2,6-dibromophenol has a pKa of 6.79. In order to obtain the desired TI close to pH 7, the use of phenols of higher pKa was necessary. In total 8 phenols were screened in the Friedel-Crafts reaction and the indicating properties of the

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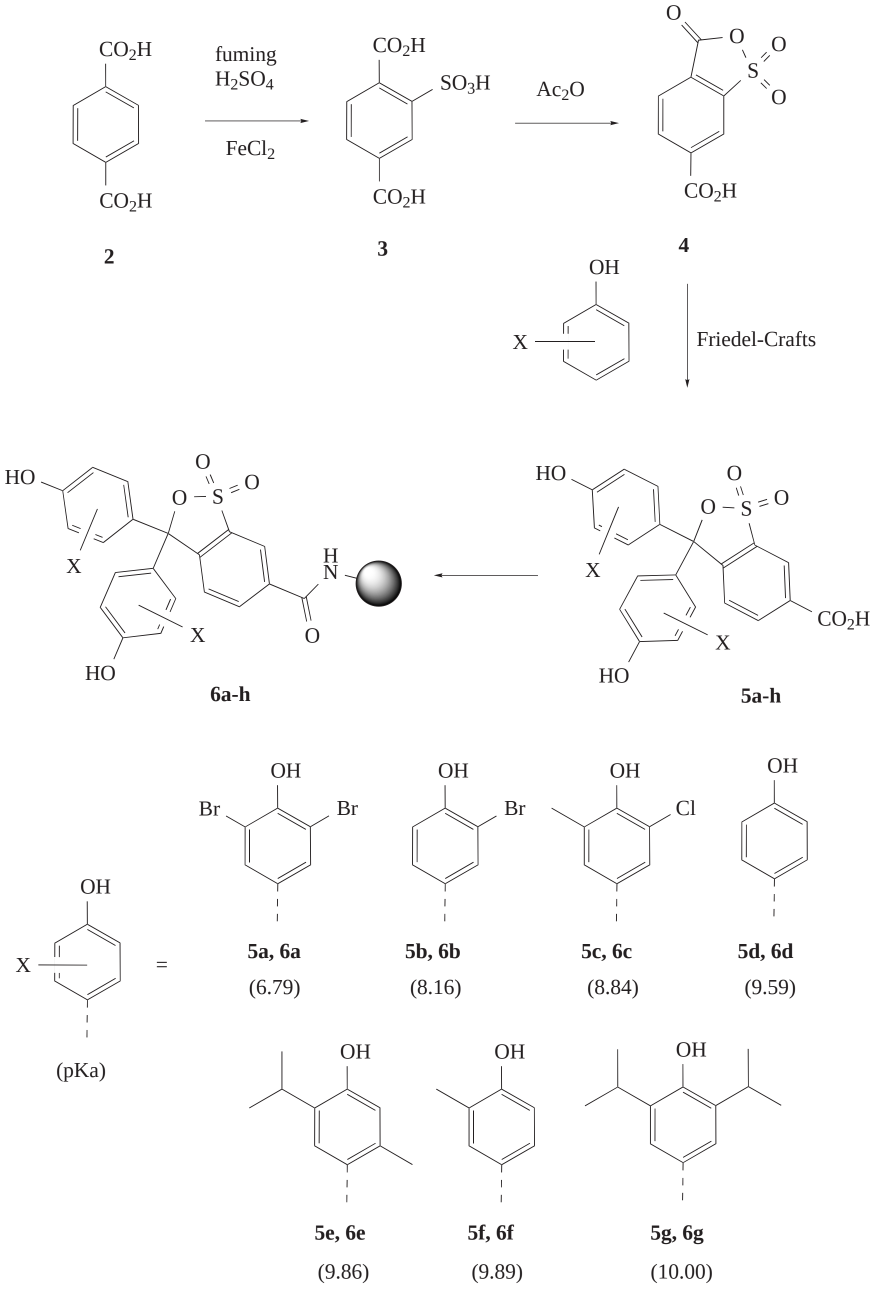
1386-2073/07 $50.00+.00

resulting crude dyes were studied. As the electron withdrawing groups on the ring deactivate the phenol toward the Friedel-Crafts reaction, the phenols of pKa lower than ca. 8.5 were practically unreactive and only very small amount of the dye **5a** and **5b** could be prepared. Although the phenols of higher pKa are more reactive, the TIs of the corresponding dyes (**5e**, **5f**, **5g**) lay in the pH range >8.0 and they did not show the required change of color at around pH 7 upon sequential treatment with acid/base. The calculated pKas of the phenols (not those of the dyes) at pH 7.4 are shown in Scheme **1**. The best compromise of the two factors could be achieved by the use of *o*-chlorocresol. This phenol has both reasonable reactivity and an appropriate pKa affording the dye **5c**. The dye **5d** prepared from simple non-substituted phenol also had an appropriate transition interval. However, because of the absence of halogen atoms, the color change was not as conspicuous as that of chlorocresol dye **5c**. The SIR **6c** was prepared by loading aminomethyl NovaGel™ resin with 0.05eq (5% of the total loading capacity) of the dye **5c**. The remaining amino groups on the resin were then capped with acetic anhydride. The color of **6c** at various pH values and also in the presence of benzylamine and pyridine is shown in Picture **1**.

The performance of the SIR **6c** as a reaction probe was examined using an amine capping reaction as the test reaction (Scheme **2**). A glass vial was charged with the solution of amine and the SIR. The SIR turned blue when mixed with the amine **7**. When the isocyanate **8** (0.9 eq) was added to the amine solution, the color of the SIR remained blue. A further portion of isocyanate **8** (0.1 eq, 1.0 eq in total) was then added. The SIR turned yellow/green color at this point. Further addition



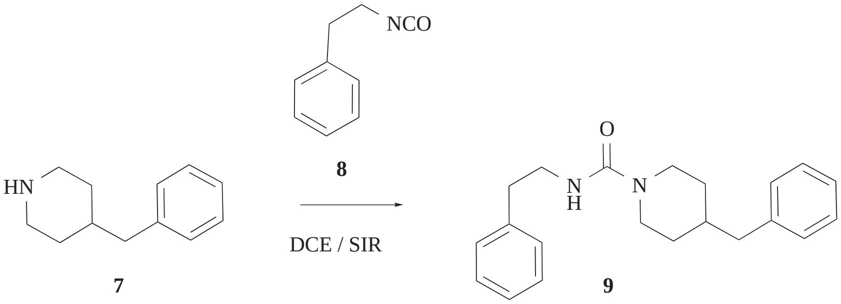
Scheme 1. Route to new SIR **6**.

of the isocyanate (0.1 eq, 1.1 eq in total) caused the color to change to yellow, and completion of the reaction was confirmed by LC-MS analysis (Table **1**).

Picture 1. SIR **6c** in buffer solution (from left pH, 5, 6, 7, 8, 9), in acetone with pyridine and (*i*-Pr)$_2$Net.

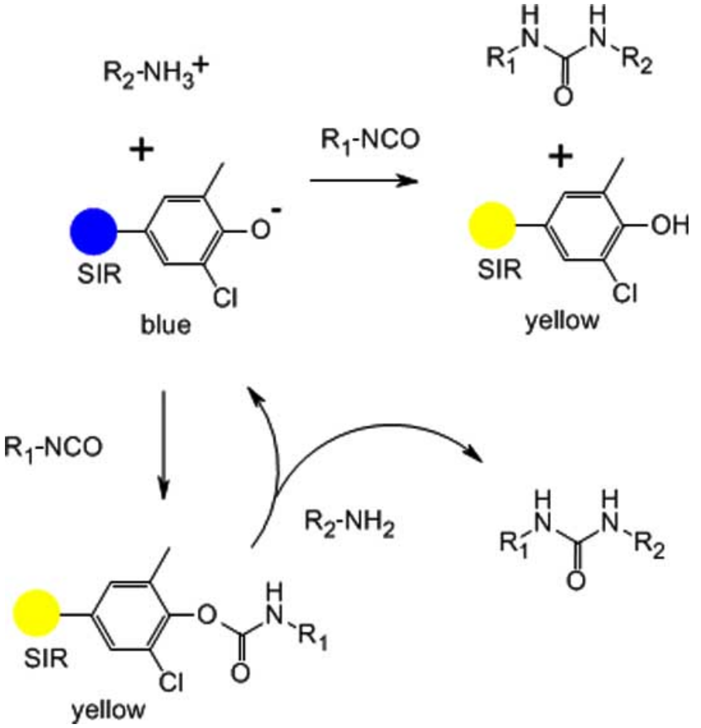
Table 1. Endpoint Indication of Amine Capping by SIR 6c

Color of 6c	Isocyanate 8 (eq)	Time (hr)	Amine 7 Consumption (%)
	0	0	0
	0.9	2	85
	1.0	4	>95
	1.1	6	100

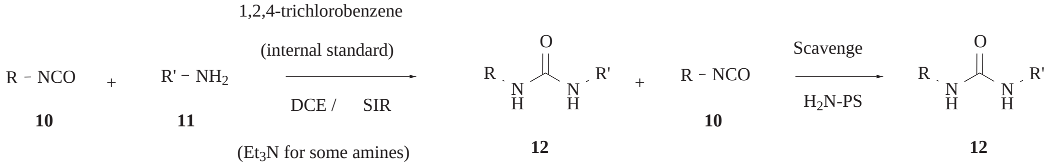


Scheme 2. Endpoint indication of amine capping by SIR **6c**.

During the study, we noticed that if more than one equivalent of the isocyanate was added in one portion, the SIR became yellow prematurely before completion of the reaction. However, the color of the SIR turned blue again usually within 30 min and turned back to yellow once again on completion of the reaction. This unexpected behavior can reaction mixture or the reaction of the amine and isocyanate be explained as follows; when excess isocyanate exists in the is relatively slow, the phenolic hydroxyl group of the chlorocresol SIR **6c** is capped to form the corresponding carbamate and thus exhibits a yellow color. The capped SIR then reacts with the amine to form the urea and regains its blue color (Scheme **3**). When all the amines are consumed, the SIR shows yellow/green neutral color indicating the completion of the reaction. If unreacted excess isocyanate exists in the



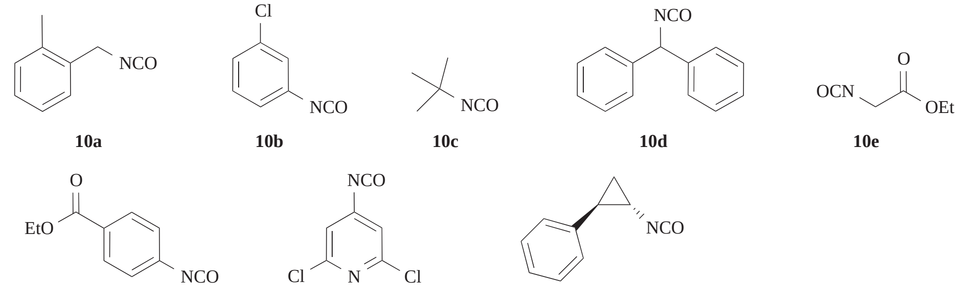
Scheme 3. Reaction of SIR **6c** and isocyanates; Catch-and-release type mechanism of urea formation.



Scheme 4. Urea library synthesis.

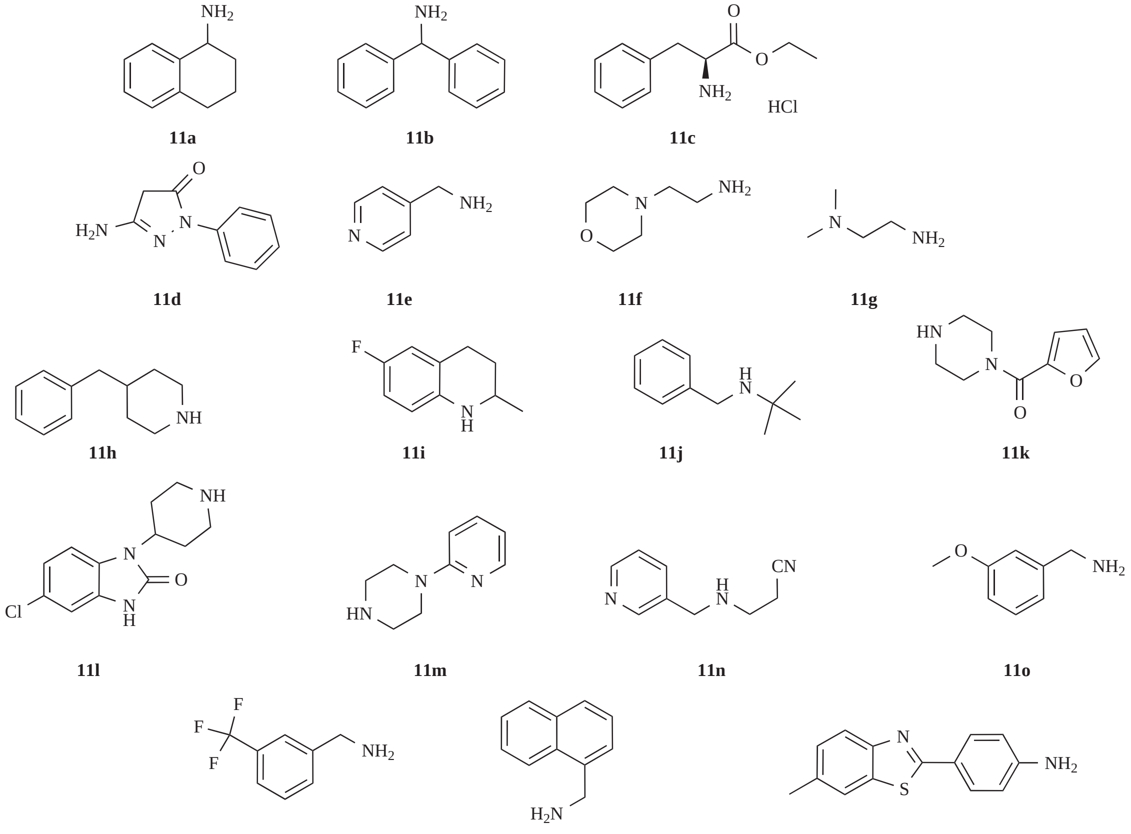
Table 2. List of Isocyanates and Amines Used in the Urea Library Synthesis

Isocyanates 10a-h



10a **10b** **10c** **10d** **10e** **10f** **10g** **10h**

Amines 11a-r



11a **11b** **11c** **11d** **11e** **11f** **11g** **11h** **11i** **11j** **11k** **11l** **11m** **11n** **11o** **11p** **11q** **11r**

mixture at this stage, the phenolic hydroxyls of the SIR are capped and thus exhibit a yellow color. This was confirmed by treating the SIR **6c** with 2-phenylethylisocyanate in the absence of amine and then adding an amine to the capped SIR. The SIR initially turned yellow. After washing the capped resin thoroughly with a solvent a solution of an amine was added. The SIR regained the blue color and formation of the corresponding urea **9** was detected by LC-MS analysis.

In order to examine the performance of the chlorocresol SIR **6c** in the reactions involving a wider variety of amine/isocyanate combinations, the synthesis of a 144 member urea library was carried out using **6c** as the endpoint indicator. The isocyanates and amines used are shown in Table **2**. Some sterically hindered, less reactive and hygroscopic substrates were included rather than selecting only the amine/isocyanate combinations that are expected to give the corresponding ureas cleanly in order to investigate the behavior of the SIR under complex circumstances.

Plates (48 well, 5 mL) were charged with chlorocresol SIR **6c** (10 mg), DCE, the amines and 1,2,4-trichlorobenzene as the internal standard. The SIR turned to blue except in the wells of 5 amines. Addition of a small amount of triethylamine was necessary for these amines as four of them were not strong enough bases to cause the SIR to turn to blue (**11d**, **11i**, **11n**, **11r**). It was surprizing that the amine **11n** was not a strong base enough to turn the SIR **6c** blue and required addition of triethylamine and the reason for this is not clear. Addition of triethylamine was also necessary where the amine was used as its hydrochloride salt (**11c**). The solutions of isocyanates (1.5 eq) in DCE were added to each well and the progress of the reaction and color of the SIR were monitored.

Most of the reactions went to near completion and the expected ureas were obtained. The images of the plates are presented in Table **4**. In Table **3**, the color of the SIR at the end of the reaction and consumption (%) of the starting amines (as determined by HPLC (NMR for the reactions of the amine **11g**)) are given. As shown in Table **4**, problematic amines could be readily detected as blue columns (Amine **11c**, **11g**, **11i**, **11n**). Some of the SIRs that turned yellow (at 30 min) regained their blue color after overnight reaction. This is consistent with the catch-and-release mechanism discussed earlier. The products in the wells of amines **11e**, **11f** could not be analyzed by HPLC/UV because of poor peak resolution. In total the contents of 128 wells were analyzed by HPLC/UV or NMR. When “success of indication” is arbitrarily defined as “SIR is yellow and amine consumption is >=90%, or SIR is blue or green and amine consumption is <90%”, the overall success rate was, 81%. Good success rates were obtained for strongly nucleophilic amines, particularly for piperidines (94%) and primary benzyl amines (94%). For the amines **11e** and **11f**, although consumption of amines could not be assessed accurately by HPLC/UV, NMR analysis of the crude product indicated that most of the reactions went to near completion. Thus, the success rate for these two columns is expected to be high. The results for the reactions carried out in the presence of triethylamine were less satisfactory. This is probably due to the fact that complete capping of the phenols of the SIRs was needed for the SIR to show yellow color in the presence of triethylamine.

Another way of analyzing these results is to focus on the failed reactions. No urea formation occurred for the reactions of the hygroscopic amine **11g** due to the moisture. The SIR clearly indicated that the reactions did not proceed to completion for this amine. It should also be noted that there were twenty amine/isocyanate combinations which gave low conversion, less than 70%, to the ureas in these experiments. The color of the SIR was found to be blue in the eighteen wells out of these twenty failed combinations, successfully detecting the low conversions in these wells.

CONCLUSION

An efficient synthetic route to a new type of self-indicating resin including the SIR **6c** has been developed. SIRs of various TIs could be prepared by the Friedel-Crafts method described herein with the limitation that dyes with TIs lower than ca. 6 could not be accessed due to the low reactivity of phenols substituted with electron withdrawing groups. The SIR **6c** has the TI centred at pH 7 and indicates the presence or absence of basic substances such as amines in the media by its conspicuous change of the color. We have also demonstrated that the SIR **6c** successfully indicated the endpoint of the reaction of amines with isocyanates in a 144-member urea synthesis. The complete and incomplete reactions could be quickly distinguished by observing the color of the SIR in the wells of the reaction plates. The SIR **6c** and also the SIRs of various TIs which can be prepared using the method described herein should prove to be highly useful for monitoring a variety of reactions in combinatorial and parallel synthesis.

EXPERIMENTAL

^{1}H and ^{13}C nuclear magnetic resonance (NMR) spectra were acquired on a Bruker Avance 400MHz spectrometer at 400.132 and 100.613 MHz respectively. Positive ion electrospray mass spectra were recorded using a Waters ZMD or ZQ mass spectrometer over the scan range 100 to 850 u in 0.5 s with a 0.1 s interscan. The electrospray capillary voltage was 2500 V and the cone voltage was 25 V. The pKas of the phenols were calculated using Marvin pKa Plug-in by ChemAxon.

Chlorocresol Sulfonephenolphthalein Dye (5c)

Anhydrous zinc chloride (0.5 eq, 2.98 g, 21.9 mmol) and 2-chloro-6-methylphenol (3.0 eq, 18.74 g, 131.5 mmol) were heated to 100°C, under nitrogen. 2-Sulfoterephthalic acid cyclic anhydride (1.0 eq, 10.0 g, 43.8 mmol) was added and the mixture heated to 140°C for 72 h. Gradually, the reaction took on a deep red color. The mixture was allowed to cool, which was then dissolved in 10% NaOH solution (220 mL). After 1 h, concentrated HCl (50-60 mL) was added carefully. The salts were precipitated by the addition to acetone (4 L) and removed by filtration. The filtrate was evaporated to a brown solid, which was purified by flash column chromatography, on silica gel, eluting with a gradient of DCM to acetone to give the title compound **5c** 3.68g (17%). ^{1}H NMR (400MHz, CD_3CN) $\delta = 8.52$ (s, 1H), 8.18 (brs, 1H), 8.34 (d, $J = 8.2$ Hz, 1H), 7.56 (d, $J = 8.2$ Hz, 1H), 8.12 (d, $J = 2.2$ Hz, 2H), 7.02 (d, $J = 1.9$ Hz, 2H), 2.18 (s, 6H); calculated mass for $C_{22}H_{16}Cl_2O_7S$: 494.00, found MS, *m/z* 495.11 $[M+H]^+$.

Sulfonephenolphthalein Dye (5d)

^{1}H NMR (400MHz, CD_3CN) $\delta = 8.58$ (s, 1H), 8.21 (d, $J = 8.2$ Hz, 1H), 7.53 (d, $J = 8.1$ Hz, 1H), 7.11 (d, $J = 8.8$ Hz, 4H), 6.80 (d, $J = 8.8$ Hz, 4H); calculated mass for $C_{20}H_{14}O_7S$: 398.05, found MS, *m/z* 399.27 $[M+H]^+$.

Sulfonephenolphthalein Dye (5e)

Calculated mass for $C_{28}H_{30}O_7S$: 510.17, found MS, *m/z* 511.32 $[M+H]^+$.

Table 3. Consumption of Amine and Color of SIR 6c[a]

	11a	**11b**	**11c**	**11d**	**11e**[b]	**11f**[b]	**11g**[c]	**11h**	**11i**	**11j**	**11k**	**11l**	**11m**	**11n**	**11o**	**11p**	**11q**	**11r**
10a	Y 100	Y 100	B 100	Y 96	Y	Y	B 0	Y 100	G 65	Y 93	Y 100	Y 100	Y 100	B 100	Y 100	Y 98	Y 96	Y 95
10b	G 100	B 100	B 100	B 36	Y	G	B 0	Y 100	B 90	Y 80	Y 100	Y 100	Y 100	B 100	Y 100	Y 100	Y 96	B 96
10c	Y 97	Y 96	B 89	B 37	Y	Y	B 0	Y 100	B 16	G 63	Y 100	Y 100	Y 100	B 100	Y 100	Y 100	Y 96	B 87
10d	Y 79	Y 80	B 96	Y 56	Y	Y	B 0	Y 100	B 56	Y 82	Y 100	Y 100	Y 100	B 12	Y 100	Y 100	Y 100	Y 93
10e	Y 100	Y 100	Y 100	Y 93	B	Y	B 0	Y 100	Y 96	Y 98	Y 100	Y 100	Y 100	B 83	Y 98	Y 100	Y 100	Y 98
10f	Y 99	B 100	B 74	Y 100	Y	G	B 0	Y 100	B 87	Y 75	Y 100	Y 100	Y 100	B 100	Y 100	Y 100	Y 100	B 79
10g	B 54	B 100	B 84	B 100	B	B	B 0	Y 100	B 94	Y 97	Y 100	Y 100	Y 100	B 92	Y 100	Y 100	Y 100	Y 57
10h	Y 100	Y 99	B 100	G 29	Y	Y	B 0	Y 100	B 73	Y 99	Y 100	Y 100	Y 100	B 100	Y 100	Y 100	Y 100	Y 93

[a] Color of the SIR 6c at the end of the reaction, B = blue, G = green, Y = yellow; Numbers are conversion (%) determined by HPLC analysis.

[b] Conversion could not be determined by HPLC/UV because of poor peak resolution.

[c] No urea formation was detected during NMR analysis of the crude sample.

Sulfonephenolphthalein Dye (5f)

^{1}H NMR (400MHz, CD_3CN) δ = 8.47 (s, 1H), 8.00 (d, *J* = 7.7 Hz, 1H), 7.24 (d, *J* = 7.7 Hz, 1H), 7.04 (s, 4H), 6.59 (d, *J* = 7.8 Hz, 2H), 1.99 (s, 6H); calculated mass for $C_{22}H_{18}O_7S$: 426.08, found MS, *m/z* 427.20 $[M+H]^+$.

Sulfonephenolphthalein Dye (5g)

Calculated mass for $C_{32}H_{38}O_7S$: 566.23, found MS, *m/z* 567.48 $[M+H]^+$.

Chlorocresol SIR (6c)

NovaGel™ aminomethyl resin (1.0 eq, 9.60 g, 6.90 mmol) was pre-swollen with DCM (50 mL). The chlorocresol dye **5c** (0.05 eq., 170 mg, 0.34 mmol) in DMF (20 mL), was added and the mixture shaken thoroughly for 5 min. PyBrOP (0.40 eq, 1.30 g, 2.80 mmol) in DMF (25 mL) and DIPEA (3.0 eq, 3.60 mL, 20.7mmol) were added and the mixture shaken overnight. The resin was washed with DMF (3 x 100mL) and DCM (3 x 100 mL) and dried under a moderate vacuum pressure. The resin was pre-swollen in DCM (70 mL). A solution of DMAP (0.05 eq, 42 mg, 0.34 mmol) in DCM (20 mL) was added followed by DIPEA (3.0 eq, 3.60 mL, 20.7 mmol) and acetic anhydride (3.0 eq, 1.95 mL, 20.7 mmol). The mixture was shaken for 6 h. The resin was washed with DCM (5 x 100 mL) and lightly dried *in vacuo*. The resin was pre-swollen in THF (70 mL). Aqueous 2M NaOH was added, and the mixture shaken for 30 min. The resin was filtered and the saponification procedure was repeated one more time. The resin was washed alternately with THF and water (5 x 100 mL), then methanol (100 mL), DCM (100 mL), TFA (10 mL) and TBME (100 mL). The resin was dried in *vacuo* to give yellow beads.

Endpoint Indication of Amine Capping by SIR/Preparation of Urea (9)

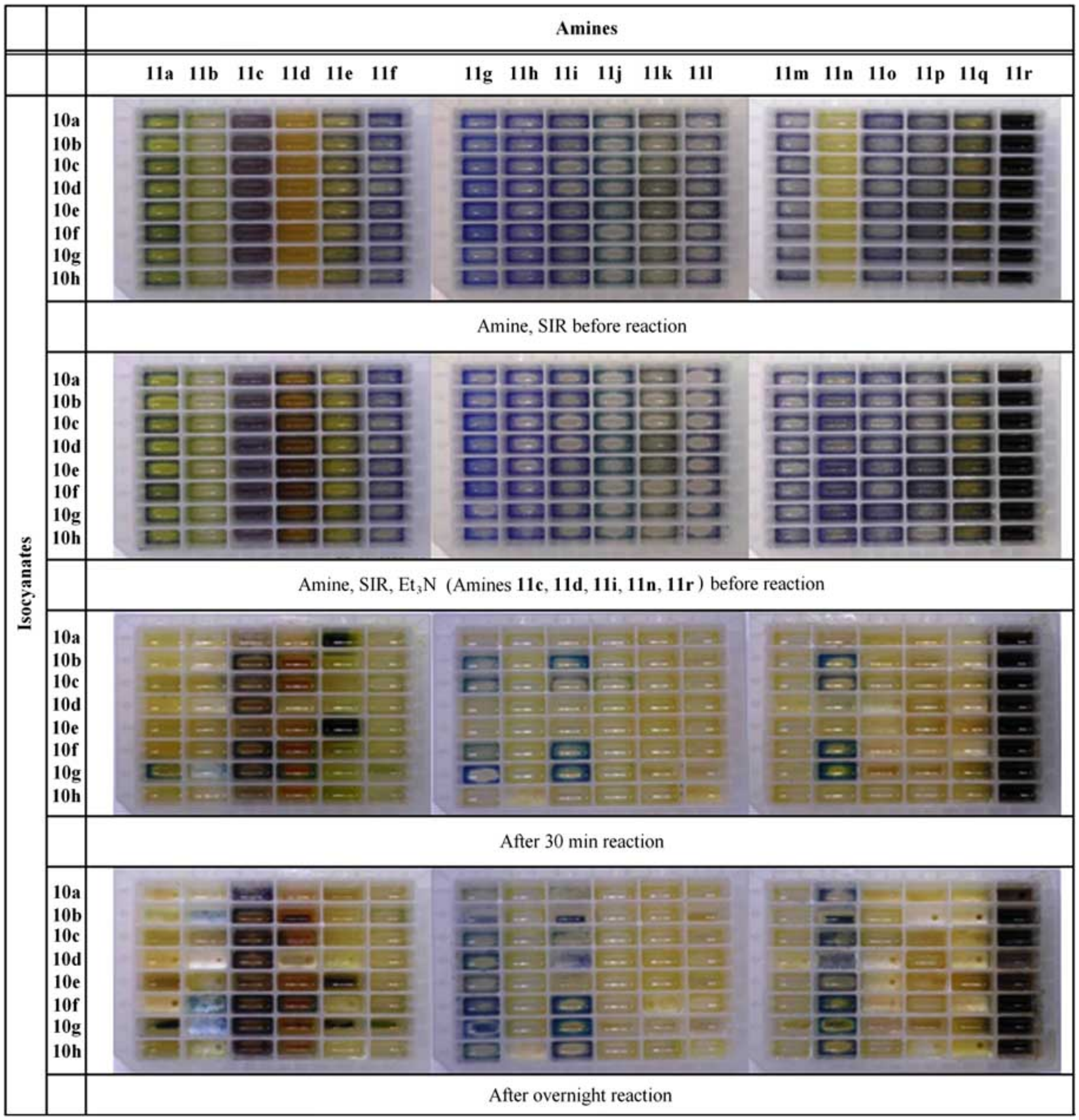
A 12 mL glass vial was charged with the amine **7** (1.0 eq, 0.80 mmol), 1,2,4-trichlorobenzene (0.30 mmol, 54 mg), SIR (10 mg) and DCE (2 mL). An aliquot (10 µL) was removed and diluted with acetonitrile/water (0.5/0.5 mL). The sample was analyzed by LC-MS. The isocyanate **8** (0.9 eq, 0.72 mmol) was added to the amine solution. After reaction for 2 h, an aliquot (10 µL) was taken and added to a mixture of aminomethyl polystyrene resin (10 mg) in HPLC grade acetonitrile (0.5 mL). The mixture was allowed to stand for 1 h. The analytical solution was filtered into a vial and the filtrate diluted with HPLC grade water (0.5 mL) for LC-MS analysis to monitor the progress of the reaction. Two further portions of isocyanate (0.1 eq, 0.08 mmol) were added to the reaction mixture, after each addition the mixture was reacted for 2 h before an aliquot was removed and analyzed in a similar manner.

The authentic sample of **9** was prepared using similar procedures starting from **7** (400 mg, 2.28 mmol); yield 590 mg (80%), ^{1}H NMR (400MHz, $CDCl_3$) δ = 7.12 - 7.32 (m, 10H), 4.44 (m, 1H), 3.84 (m, 1H), 3.81 (m, 1H), 3.47 (dd, J = 12.6 Hz, 6.8 Hz, 2H), 2.81 (t, J = 6.8 Hz, 2H), 2.62 (t, J = 12.8 Hz, 2H), 2.52 (d, J = 6.9 Hz), 1.61 (brd, J = 7.0 Hz, 2H), 1.60 - 1.72 (m, 1H), 1.08 - 1.20 (m, 2H); ^{13}C NMR (100MHz, $CDCl_3$) 157.6, 140.1, 139.5, 129.1, 129.0, 128.5, 128.3, 126.3, 126.0, 44.2, 43.1, 42.1, 38.1, 36.4, 31.9; calculated mass for $C_{21}H_{26}N_2O$: 322.20, found MS, *m/z* 323.41 $[M+H]^+$.

Endpoint Indication of 8 X 18 Amine Capping Reaction Using SIR 6c

A solution of 1,2,4-trichlorobenzene in DCE (0.12 M) was used to make up solutions of each of 18 amines **11a-r** (9.6 mmol in 42 mL). Solutions of each of the isocyanates **10a-h** (17.8 mmol) were made up in DCE (9.9 mL). Each well in 3 x 48 well, 5 mL plates were charged with chlorocresol SIR **6c** (10 mg) and the color noted. To each of the wells, the relevant amine solutions (1.0 eq, 2.5 mL, 0.60 mmol) were added. Triethylamine (3.0 eq, 0.25 mL, 1.8 mmol, for amine **11c**; 0.5 eq, 40 µL, 0.3 mmol, for amine **11d**, **11i**, **11n** and **11r**) was added to each well of amine **11d**, **11i**, **11n** and **11r**. An aliquot

Table 4. Color of SIR 6c in Urea Library Synthesis



(20 μL) from each well was taken and analyzed by LC-MS. To each of the wells in the 48 well plates, the relevant isocyanates (1.5 eq, 0.5 mL, 0.90 mmol) were added. The plates were gently agitated for 6 h, and an aliquot (20 μL) was removed from each well for LC-MS analysis. The plates were gently agitated overnight, and another aliquot (20 μL) was removed from each well for LC-MS analysis. The plates were filtered into 12 mL glass racked vials containing aminomethyl resin (1.6 mmol, 0.8 g per well). DCE (6 mL, and 0.5 mL of DMF in some cases) was added, and the reactions were allowed to stand over night. The resins were filtered off, and the filtrate transferred to 5 mL 48 well plates and evaporated to dryness. The resins were washed twice with methanol and the filtrates added to the plates, each time being evaporated to dryness, giving the crude products.

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Received: December 21, 2006 **Revised: February 27, 2007** **Accepted: March 6, 2007**