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Automated chemical synthesis: chemistry development on the Nautilus 2400™

John A. Porco, Jr.,* Tracy L. Deegan, Wayne Devonport, Owen W. Gooding, Jeff W. Labadie, Alasdair A. MacDonald, William S. Newcomb and Paul van Eikeren

*Argonaut Technologies, 887 G Industrial Rd., San Carlos, CA 94070, USA. *Correspondence*

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Introduction

The automation of the organic synthesis of small molecules is now of major focus in the pharmaceutical industry (1). Over the last few years, commercially available instrumentation for automated organic synthesis has steadily increased, concomittant with the need for increased throughput for chemistry development and library synthesis (2). However, it is apparent that further development of robust and inert instrumentation is required to facilitate the automation of a broad range of synthetic organic chemistries, either on solid-support or using conventional solution-phase methods. In this article we will describe the Nautilus 2400™, a chemistry development workstation, and demonstrate examples of its ability to handle air- and moisture-sensitive reagents, to exhibit precise control over reaction time, and to perform individual temperature control in segregated reaction vessels. The result is a useful tool for drug discovery which allows chemists to increase efficiency in chemistry development, lead optimization and library synthesis.

The Nautilus 2400™, a chemistry development workstation

New instrumentation for automated chemical synthesis should allow chemists to use the broadest range of reaction conditions, as well as allow maximum flexibility (*e.g.*, temperature, time control, *etc.*) in their syntheses. In the design phase of a chemistry development platform, we worked closely with medicinal chemists to determine the features and specifications that were required for general chemical synthesis. The result of the design efforts was the Nautilus 2400™ automated chemistry development workstation (Fig. 1). Unlike robotic platforms, the Nautilus is a closed, pressurized system where individual reaction vessels are kept under an inert atmosphere of either nitrogen or argon gas. In order to handle the range of reagents and solvents typically used in the organic synthesis laboratory, all wetted surfaces in the Nautilus are composed of either Teflon® or glass. Reagents are stored and delivered from two compartments: an autosampler (AS) for "diversity" reagents and a reagent solvent enclosure (RSE) for bulk reagents and solvents. Fluids are transported through the system by pressurized gas (approximately 20 psi for reaction vessels and 10 psi for RSE bottles). Reagents and solvents are delivered to 24 (3 modules of 8) transparent glass reaction vessels (8, 15 or 23 ml) which permit a visual assessment of the reaction at any point in the synthesis. The temperature of individual reaction vessels is independently regulated from -40 °C to 150 °C by a combination of chilled gas and controlled heating. Reaction mixing is accomplished by a rocking agitator that rocks each bank of 8 vessels through an arc of 217°, which generates efficient turbulent mixing and minimizes damage to resin beads. These unique hardware capabilities are coordinated by a computer through a proprietary graphical user interface (GUI) software system which employs the industry standard SQL database, Microsoft Access™. The result is a powerful tool for chemistry development, lead optimization and trial library synthesis.

Use of air-sensitive reagents on the Nautilus

We have utilized a broad range of air- and moisture-sensitive reagents on the Nautilus 2400™ in the course of conducting chemistry development studies and multistep syntheses. Introduction of bulk reagents of this type on the instrument as solutions via syringe or cannulation is facilitated by the use of reagent bottles that contain a sin-

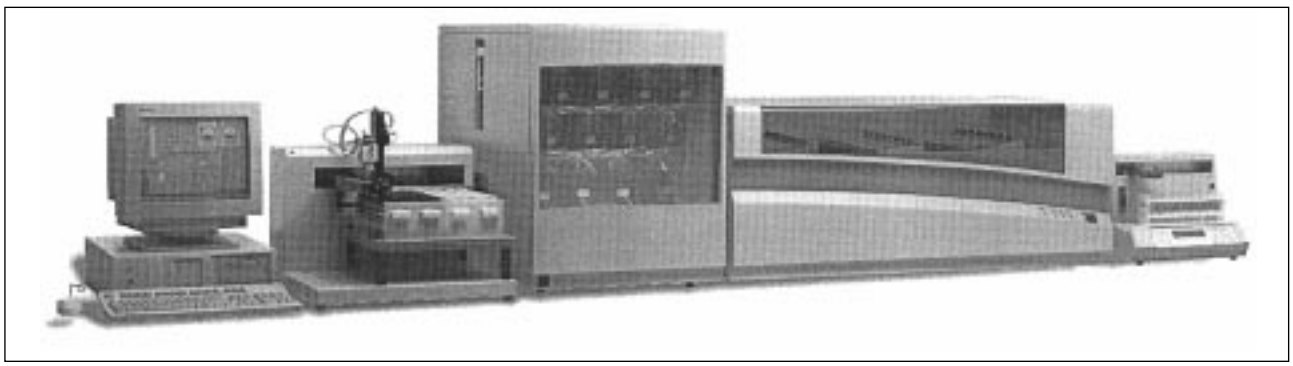


Fig. 1. The Nautilus 2400: a modular organic synthesis workstation.

gle teflon-lined septum port (Fig. 2). Some of the more reactive reagents delivered and utilized include: *n*-butyllithium (*n*-BuLi), lithium diisopropylamide (LDA), potassium hexamethyldisilazide (KHMDS), Grignard reagents, tributylphosphine, Lewis acids (*e.g.*, 1.0 M $TiCl_4$ in toluene), and transition metal catalysts (*e.g.*, $Pd(PPh_3)_4$ in dioxane). Notably, Pd(0) stock solutions can be stored on the Nautilus for days, without noticeable precipitation of palladium black, illustrating the high level of inertness of the Nautilus closed system.

As an example of the utilization of air- and moisture-sensitive reagents on the Nautilus, we performed solution-phase parallel synthesis of isoxazoles (in collaboration with T.J. Nitz and Diane Cebzanov of Viropharma, Inc., Malvern, PA). The experimental procedure involved the addition of keto oxime dianions to esters at low temperature, followed by acid catalyzed cyclization/dehydration (3, 4). In this case, we used the Nautilus to correlate the effect of reaction temperature and oxime dianion stoichiometry on the distribution of products, in order to effect chemoselective addition of the dianion to the ethyl ester of compound (**1**) and thus avoid addition to the benzonitrile (Fig. 3). Solutions of dilithium acetone oxime (0.4 M in THF) were prepared off-line (acetone oxime, 2 equiv.

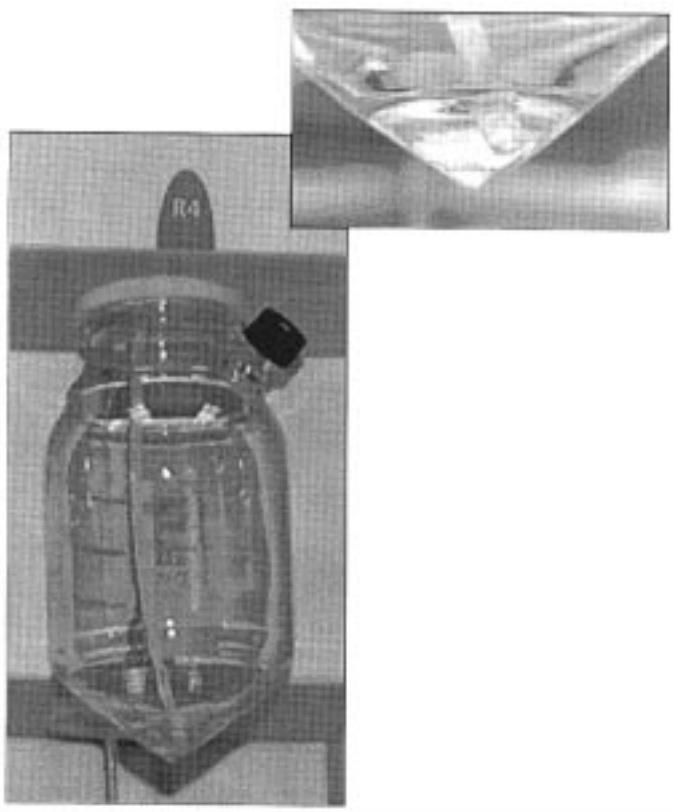
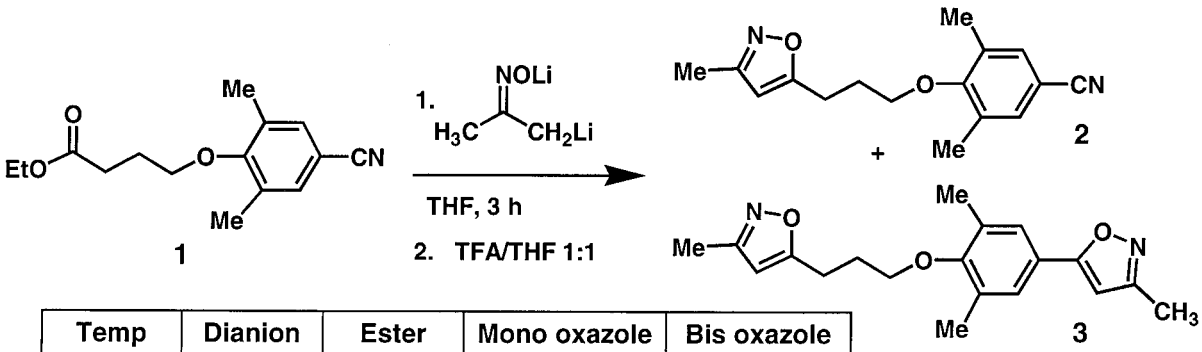


Fig. 2. Reagent bottles for introduction of air-sensitive reagents on the Nautilus.



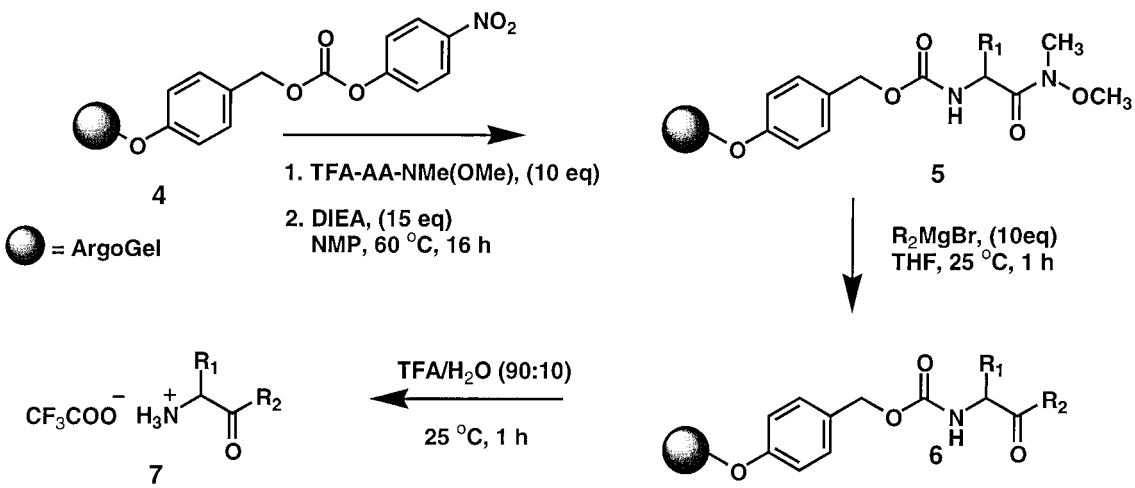
Temp	Dianion (eq)	Ester (mol%)	Mono oxazole (mol%)	Bis oxazole (mol%)
- 10 ºC	1.6	12	77	11
- 10 ºC	2.0	0	54	46
- 20 ºC	1.6	9	79	12
- 20 ºC	2.0	0	79	21

Fig. 3. Reaction optimization on the Nautilus: isoxazole synthesis.

BuLi, THF, 0 ºC) and added by cannula via a septum port bottle on the RSE location of the instrument. Temperature and oxime dianion stoichiometry were varied. The amounts of mono-isoxazole product (**2**) and bis-oxazole byproduct (**3**) were determined by integration of the oxazole protons in the NMR spectra. Results of the experiments, representative examples of which are shown in Figure 3, illustrate how the Nautilus was used to determine the effect of temperature and stochiometry on the yield and chemoselectivity of a synthetic transformation. It is also significant that we were able to effectively deliver the highly reactive and moisture-sensitive acetone oxime dianion solution in these experiments, since protonation of the oxime dianion by adventitious water or traces of protic solvent in the delivery lines would have led to precipitation of salts and blockage of the lines, which was not observed.

As a further example of an application employing moisture-sensitive reagents on the Nautilus, we have automated the addition of Grignard reagents to Weinreb amide derivatives on solid-support (Scheme 1) (5, 6) in collaboration with Drs. Scott Berk and Kevin Chapman (Merck, Rahway NJ). ArgoGel-Wang resin (7) (0.43 mmol/g) was converted on large scale to the nitrophenyl

Scheme 1: Automated Synthesis of Ketones via Weinreb Amides



carbonate derivative (**4**) using standard conditions (8). Resin (**4**) was weighed into reaction vessels and treated with the TFA salts of *N*-methoxy-*N*-methyl amides derived from amino acids (9) and excess diisopropylethylamine (DIEA) in *N*-methylpyrrolidinone (NMP) at 60 °C for 16 hours. The resin-bound carbamates (**5)** were washed extensively with dimethylformamide to remove byproducts, re-equilibrated in dry THF and treated with Grignard reagents (10 equiv., 25 °C, 1 h). At the end of this time, the reaction vessels were drained of excess Grignard reagent, washed with dry THF and finally quenched with glacial acetic acid to form the keto-carbamate derivatives (**6**). After further washing to remove excess salts, the corresponding amino ketone products (**7**) were cleaved from the resin (90:10 TFA/water), lyophilized and analyzed by HPLC. As shown in Table I, chemical yields and purities for the products were high and generally in the 80-90% range.

As a further illustration of the use of air- and moisture-sensitive reagents, and the capabilities of the Nautilus to facilitate chemistry development, we carried out the Mitsunobu reaction of sulfonamides on solid support, in collaboration with Drs. Sharon Dankwardt and David B. Smith (Roche Biosciences, Palo Alto, CA) (10). The alkylation of sulfonamides under Mitsunobu conditions has been previously reported in solution employing 1,1-(azodicarbonyl)dipiperidine (ADDP) or 1,1-azobis-(*N,N*-dimethylformamide) (TMAD) with tributylphosphine (PBu_3) in benzene (11, 12). In order to optimize conditions for the Mitsunobu alkylation of amino acid-derived sulfonamides on solid-support (see Fig. 4), we investigated the effect of resin selection (Polystyrene-Wang *vs*. ArgoGel Wang), reaction solvent (toluene or dichloromethane (DCM)) and azo reagent (ADDP or TMAD).

ArgoGel and Polystyrene Wang resins (Bachem) were coupled with Fmoc glycine or Fmoc phenylalanine (1,3-diisopropylcarbodiimide (DIC), DCM, cat. 4-dimethylaminopyridine, (DMAP)). The resulting resins were deprotected (20% piperidine/DMF) and treated with an aromatic sulfonyl chloride derivative ($ArSO_2Cl$, DIEA, DCM, 12 h) to afford alkylation substrates (**8**). Automated Mitsunobu reactions were performed by addition of 6 equiv. PBu_3 (1 M in the indicated solvent system, delivered from the RSE) to the resin followed by 6 equiv. of the alcohol (1 M in THF). The reaction vessels were cooled to -10 °C, then 6 equiv. of the azo reagent (1 M in DCM) was added from the RSE. The reactions were warmed to room temperature and agitated for 12 hours. After a washing regimen, alkylation products (**9**) were cleaved from the resin with 50% TFA/DCM, concentrated and analyzed by HPLC.

Data from an automation study comparing the effect of resin selection on the course of the Mitsunobu alkylation is given in Figure 4. It is apparent that Mitsunobu alkylations achieve higher levels of conversion on ArgoGel Wang relative to Polystyrene Wang resin. This may be due to the greater mobility and "solution-like" environment of molecules that are bound to the end of the PEG chains of ArgoGel-Wang relative to molecules near the polystyrene backbone. Figure 5 shows results correlating solvent and azo reagent selection with extent of conversion for alkylation of ArgoGel Wang resin-derived sulfonamides. Evidently, the reaction performs better in toluene *versus* DCM and works well with either azo reagent.

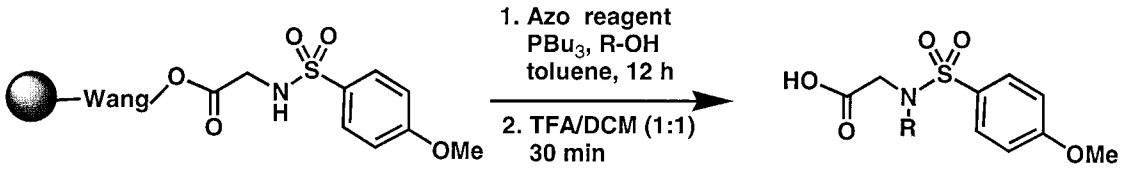
Using the "best" conditions (ADDP as azo reagent and toluene as solvent), we utilized ArgoGel Wang-supported sulfonamides to prepare a trial library of *N*-alkylsulfonamides on the Nautilus. A small library of 16 compounds was prepared using two different amino acids, four different alcohols and two aromatic sulfonyl chlorides (Fig. 6). From the results, it is apparent that α-substituted amino acid-derived substrates work equally well as unsubstituted (glycine)-based derivatives. However, sterically hindered alcohols (*e.g.*, cyclohexylmethanol) are slower substrates for Mitsunobu alkylation as evidenced by low conversions to alkylated sulfonamides. This example demonstrates the utility of the Nautilus for both chemistry development and subsequent synthesis of a trial library of compounds.

Individual time control

Rigorous control over the reaction time of each vessel during chemistry development is frequently needed. Such reaction scheduling is readily achieved on the Nautilus 2400™ by employing the "controlled reaction time" procedure in the system software. This function allows for a reaction to be quenched after a programmed period of time from initiation. This capability is demonstrated using Suzuki biaryl synthesis (13) as an example (Fig. 7). ArgoGel-rink-Fmoc was deprotected (20% piperidine/DMF) and acylated with 2-iodobenzoic acid under

Table I: Amino ketone products: % yields and (% purity).

R2→ R1↓	-Benzyl	-Phenyl	4-Fluorophenyl	m-Tolyl
-Methyl	90.4 (96.1)	83.0 (98.5)	90.9 (97.2)	93.6 (97.4)
-Isopropyl	83.9 (96.6)	83.7 (94.3)	87.0 (92.5)	93.9 (94.2)
-Benzyl	82.9 (97.6)	92.9 (97.3)	91.7 (97.0)	93.4 (98.9)



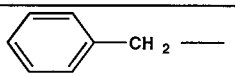
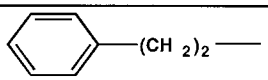
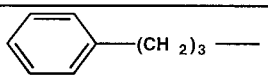
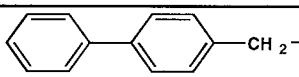
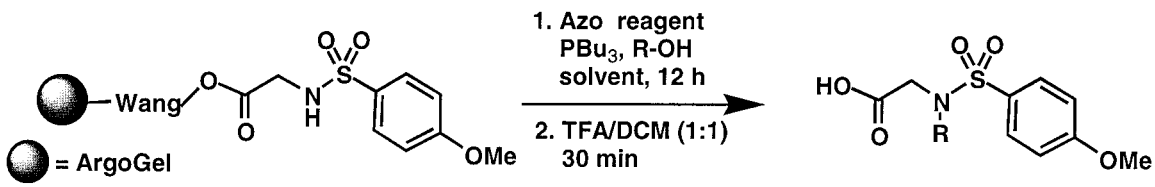
		Conversion (%)	
R-	Azo Reagents	Polystyrene	ArgoGel
	ADDP	81	88
	ADDP	73	76
	ADDP [TMAD]	77 [66]	92 [87]
	ADDP [TMAD]	88 [87]	100 [100]

Fig. 4. Mitsunobu alkylation: effect of resin selection.

standard coupling conditions (DIC/HOBT) to provide the resin-bound iodobenzamide (**10**). In a typical run, 200 mg (0.074 mmol) of resin was placed in 8-ml reaction vessels and subjected to a resin washing protocol with ethylene glycol dimethyl ether (DME). A solution of $PdCl_2(PPh_3)_2$ (0.017 M) in NMP was then delivered, followed by delivery of boronic acid solutions (2-thiophene or *o*-tolyl, 0.6 M in EtOH) from the autosampler. Concentrated aqueous salt solutions (*e.g.*, 2.0 M sodium carbonate) were then delivered from the RSE. In this example, coupling times of 1, 3, 6 and 10 hours were programmed using a controlled reaction time procedure at a reaction temperature of 90 °C. At the end of the programmed reaction time, the selected vessels were cooled down and the resin in the vessels washed. After the final reaction time had been completed, all reaction vessels were concurrently washed



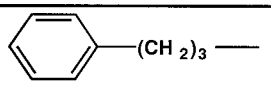
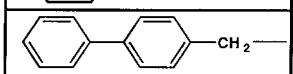
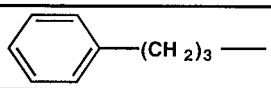
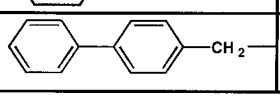
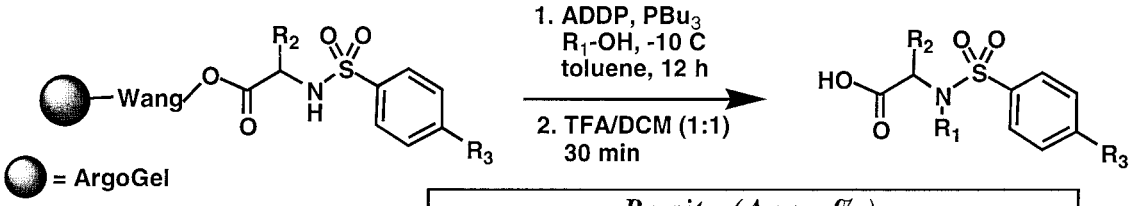
		Conversion (%)	
R	Azo Reagent	CH_2Cl_2	toluene
	TMAD	83	87
	TMAD	100	100
	ADDP	74	92
	ADDP	97	100

Fig. 5. Mitsunobu alkylation: effect of solvent and azo reagent selection.



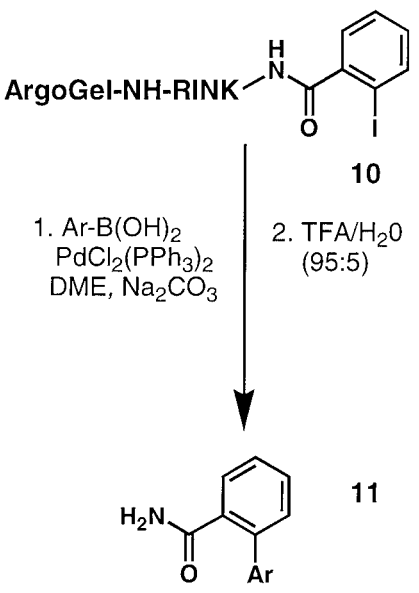
	Purity (Area %)			
	R_2 = -H		R_2= -CH_2Ph	
R_1	R_3=-OCH_3	R_3=-CH_3	R_3=-OCH_3	R_3=-CH_3
-CH_2-	31	48	31	35
-CH_2-	88	89	92	96
-$(CH_2)_2$-	76	82	88	88
-$(CH_2)_3$-	80	81	80	90

Fig. 6. Mitsunobu alkylation: trial library on the Nautilus 2400.

and cleaved (95:5 TFA/water, 25 °C, 0.5 h) to afford biaryl benzamide derivatives (**11**). Figure 7 indicates that reactions were generally complete on the *o*-iodobenzamide system in 1 hour, with the more sterically hindered *o*-tolyl boronic acid requiring about 3 hours for complete coupling. Longer reaction times also gave decreased yields as evidenced by the material balance of reactions (Fig. 7). The controlled reaction time procedure is easily modified, allowing flexibility in what is executed by the instrument (*e.g.*, quench, number and types of washes, *etc.*) at the end of each programmed time.

Individual temperature control

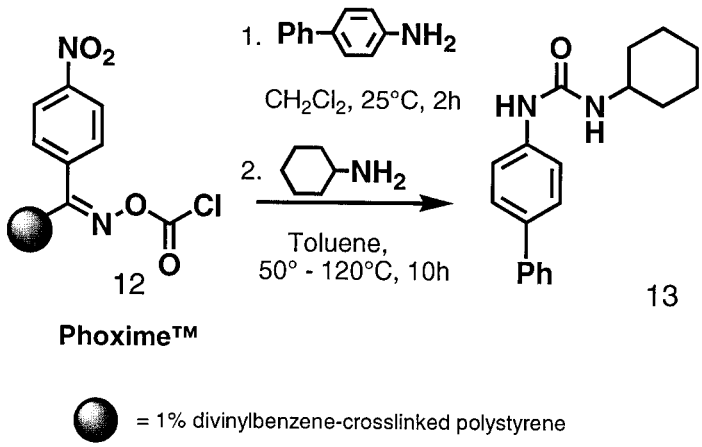
Another feature of the Nautilus is the ability to specify and maintain individual reaction temperatures for each of the 24 reaction vessels on the instrument. In collaboration



Boronic acid	Time	Product (Area%)	Material Balance (mol%)
2-thiophene	1 h	100	90
o-tolyl	1 h	90	99
2-thiophene	3 h	100	95
o-tolyl	3 h	98	100
2-thiophene	6h	99	91
o-tolyl	6h	98	96
2-thiophene	10 h	100	67
o-tolyl	10 h	99	62

Fig. 7. Biaryl formation: controlled reaction time.

Scheme 2: Solid Phase Synthesis of Cyclohexyl-4-Biphenyl Urea



with Drs. Mark Scialdone and Steve Shuey (Dupont Central RD, Wilmington, DE), we used a temperature matrix on the Nautilus to determine the optimal temperature for thermolysis of a polymer-bound oxime carbamate and subsequent urea formation. Polymer-bound oxime carbamates derived from the *p*-nitrophenyl(polystyrene)-ketoxime chloroformate resin (**12**) (14) (Phoxime™) (15) have been shown to serve as latent isocyanates upon thermolysis (16). As a case study, we examined the dependence of the isolated yield of cyclohexyl-4-biphenyl urea **(13)** on the thermolysis temperature of a polymer-bound oxime carbamate.

Phoxime™ resin (**12**) (200 mg, 0.15 mmol) was placed in each of eight, 8-ml reaction vessels on the Nautilus. Three equiv. of 4-biphenylamine in dichloromethane (DCM) were added to each reaction vessel and the reactions were agitated at 25 °C for 2 hours to afford the resin-bound carbamate intermediate (Scheme 2). After washing, the oxime carbamate resins were heated in toluene at 50-120 °C (10 °C increments) for 10 hours in the presence of 4 equiv. of cyclohexylamine. Products were concentrated to afford urea (**13**) in high chemical yields (15). In this instance, optimal thermolytic cleavage conditions appear to be above 80 °C as shown in Figure 8. Additionally, inspection of the IR spectrum of the recovered oxime resin from the higher temperatures indicated complete cleavage of the polymer-supported oxime carbamate (CO stretch: 1750 cm^{-1}). Temperature control of this type is useful for chemistry development, especially when combined with variations in reaction solvent and reaction time.

Conclusions

The results described herein demonstrate that the Nautilus 2400™ is a useful tool which synthetic chemists can use to develop chemistry, optimize lead compounds, resynthesize leads and prepare trial libraries of compounds. The Nautilus' inert, closed system and its compatibility with a wide variety of sensitive reagents, combined with features such as individual temperature control

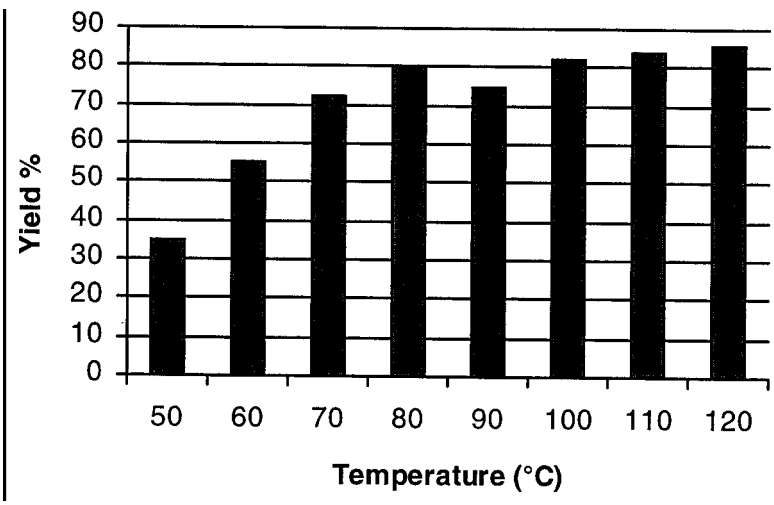


Fig. 8. Urea yield as a function of temperature.

of reaction vessels and controlled reaction time, make the instrument a powerful development tool. However, there is still a great need to develop other types of automated synthesis equipment, including those designed specifically for high-throughput library synthesis, process development and manual synthesis applications. Further studies on the use of the Nautilus 2400™ for chemistry development and new developments related to instrumentation for organic synthesis will be the subject of future reports from this laboratory.

Acknowledgements

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