Organic Process Research & Development **2010,** *14,* 1364–1372

A Mechanistic Insight into a Simple C−N Bond Formation via S_N2 Displacement: A Synergistic Kinetics and Design of Experiment Approach

Luca Massari,[†,*] Laura Panelli,[‡] Mark Hughes,[§] Federica Stazi,[†] William Maton,[†] Pieter Westerduin,[†] Federico Scaravelli,[†] and Sergio Bacchi[†,*]

Chemical Development, Synthetic Chemistry, GlaxoSmithKline Medicine Research Centre, Via Fleming 4, 37135 Verona, Italy, Chemical Development, Particle and Process Science and Engineering, GlaxoSmithKline Medicine Research Centre, Via Fleming 4, 37135 Verona, Italy, and Continuous Processing, Particle and Process Science and Engineering, GlaxoSmithKline, Gunnels Wood Road, Stevenage SG1 2NY, U.K.

Abstract:

A novel series of 1,2,4-triazol-3-yl-azabicyclo[3.1.0]hexanes was recently identified as new highly potent and selective dopamine (DA) D_3 receptor antagonists. This class of molecules deserved the Chemical Development special attention to quantify the reliability and robustness of the pivotal S_N2 displacement step between the 1,2,4-triazol-3-yl-halide derivative (4) and variously substituted azabicyclo[3.1.0]hexanes (5). To reach this goal we applied the classical Design of Experiment (DoE) approach, simultaneously trying to build up a descriptive kinetic model of the chemistry. The synergistic use of these two techniques allowed us to select new, higher-yielding and more robust reaction conditions and, at the same time, to identify their Design Space.

Introduction

Scientists at GlaxoSmithKline (GSK) recently discovered a series of novel 1,2,4-triazol-3-yl-azabicyclo[3.1.0]hexanes, potent and selective modulators of dopamine (DA) D_3 receptors.[1] This new class of compounds has potential for the treatment of drug addiction, wherein antagonism of the D_3 receptor might be beneficial.[2] An alternate take on dopamine must stabilise DA levels in order to disconnect the linkage between drug use and dopaminergic reward. A mouse study using the D3 antagonist SB-277011 (from GSK) found that D3 antagonism was superior to that of naltrexone or acamprosate in reducing alcohol self-administration. The consequence of this exciting piece of science was the need to rapidly enter in Chemical Development with a substantial number of New Chemical Entities.

It is common understanding that the objective of process development is simply to deliver drug substances. While this can be viewed as the output, the input of Chemical Development is the research of robust synthetic procedures. In order to quantify the reliability of a manufacturing process the ICH Q8 Guidance[3] has introduced the key-concept of Design Space. The ICH Q8 defines the "Design Space" as "the multidimensional combination and interaction of input variables and process parameters that have been demonstrated to provide assurance of quality". The actual construction of a Design Space requires a quantitative methodology to simultaneously incorporate the following: correlation among process responses (attributes) at each fixed operating condition, model parameter uncertainty, many sources of input and process variation, and a measure of assurance for meeting process specifications. The intention of this paper is to provide an overview of how we manage to deliver the Design Space of the key S_N2 displacement step between the 1,2,4-triazol-3-yl-halide derivative and variously substituted azabicyclo[3.1.0]hexanes for the reliable synthesis of a novel class of selective dopamine DA D_3 receptor antagonists.

Discussion

The pivotal stage in the synthesis of the 1,2,4-triazol-3-yl-azabicyclo[3.1.0]hexanes (**1**) was a nucleophilic displacement of the primary alkyl halide 3-chloropropylthio-triazole (**4**) by the aryl azabicyclo[3.1.0]hexane derivative (**5**) (Scheme 1).

The synthesis of the alkyl halide started with the commercially available 4-methyl-1,3-oxazole-5-carboxylic acid (**2**) which was subjected to amidation with the 4-methyl-3-thiosemicarbazide by means of the coupling agent T3P (propane phosphonic acid anhydride) as a solution in ethyl acetate. The resulting thioamide was dehydrocyclised using an organic base and the subsequent thiotriazole derivative **3** was almost regio-

* To whom the correspondence should be addressed. Private e-mails: giobacchi@hotmail.com, luca.m.massari@googlemail.com.

† Chemical Development, Synthetic Chemistry, Italy.

‡ Chemical Development, Particle and Process Science and Engineering, Italy.

§ Continuous Processing, Particle and Process Science and Engineering, U.K.

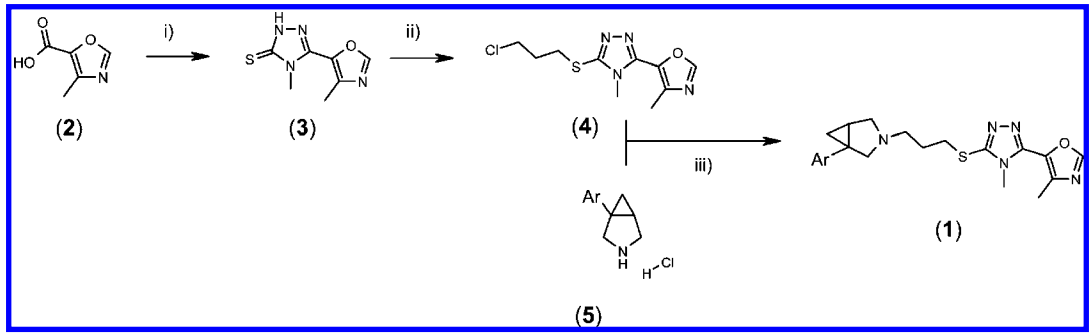
(1) Micheli, F.; Arista, L.; Bonanomi, G.; Blaney, E. F.; Braggio, S.; Capelli, A. M.; Checchia, A.; Damiani, F.; Di-Fabio, R.; Fontana, S.; Gentile, G.; Griffante, C.; Hamprecht, D.; Marchioro, C.; Mugnaini, M.; Piner, J.; Ratti, E.; Tedesco, G.; Tarsi, L.; Terreni, S.; Worby, A.; Ashby Jr, C. R.; Heidbreder, C. *J. Med. Chem.* **2010**, *53*, 374–391.

(2) (a) Arista, L.; Bonanomi, G.; Capelli, A. M.; Damiani, F.; Di-Fabio, R.; Gentile, G.; Hamprecht, D.; Micheli, F.; Tarsi, L.; Tedesco, G.; Terreni, S. PCT Int. Appl. WO/2005/080382, 2005. (b) Bonanomi, G.; Checchia, A.; Fazzolari, E.; Hamprecht, D.; Micheli, F.; Tarsi, L.; Terreni, S. PCT Int. Appl. WO/2006/108701, 2006. (c) Arista, L.; Cardullo, F.; Cecchia, A.; Hamprecht, D.; Micheli, F.; Tedesco, G.; Terreni, S. PCT Int. Appl. WO/2006/133946, 2006.

(3) The ICH Guidance Q8 represents the U.S. Food and Drug Administration (FDA) current thinking on Pharmaceutical Development manufacturing process. This guidance describes the suggested contents to input in a regulatory submission providing an opportunity to present the knowledge gained through the application of scientific approaches and quality risk management to the development of a product and its manufacturing process. This guidance is part of a set of Guidance for Industry developed within the Expert Working Group (Quality) of the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH). ICH Q8 Pharmaceutical Development, (R2); U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER): Rockville, MD, Aug 2009.

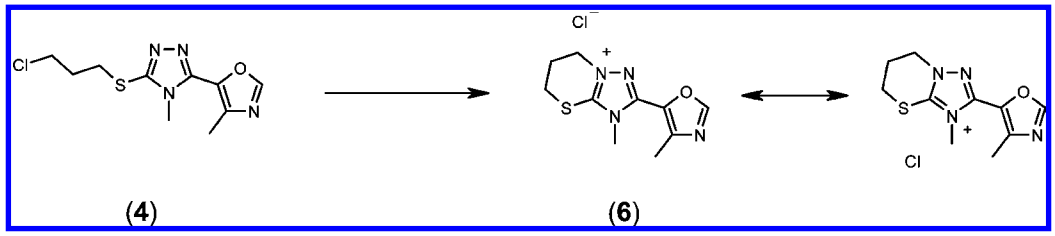
10.1021/op100176u
Published on Web 10/04/2010

Scheme 1. **Synthesis of 1,2,4-triazol-3-yl-azabicyclo[3.1.0]hexanes (1)[a]**

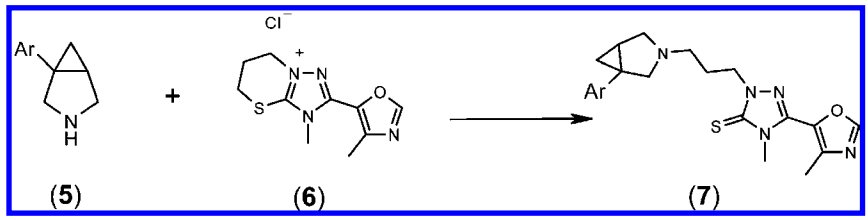


[a] Reagents and conditions: (i) 4-methyl-3-thiosemicarbazide, T3P in ethyl acetate, TEA; (ii) 1-bromo-3-chloropropane, K_2CO_3, MeOH, acetone; (iii) DMSO, KI, TEA.

Scheme 2. **Synthesis of the cyclised derivative (6)**



Scheme 3. **Synthesis of the *N*-alkylated derivative (7)**



selectively alkylated at the sulphur atom adding 1-bromo-3-chloropropane in a mixture of acetone and methanol in presence of a base. The last bond-forming step was the formation of a C−N bond via S_N2 displacement of a chlorine atom by the aryl azabicyclo[3.1.0]hexane (**5**).[1,4] The chemistry was catalysed by potassium iodide, and triethylamine was used to both free the secondary amine, which was added as a hydrochloride salt, and to neutralise the HCl produced during the coupling. This stage was of pivotal importance for the control of the impurity profile, not only because it generated the skeleton of the dopamine's modulator but also because it gave rise to a number of byproducts. Among them, two caused the most concern, the positively charged derivative formed via the intramolecular cyclisation of the chloropropylthiotriazole derivative **6**, typically present in 20−25% a/a by HPLC (Scheme 2) and the compound due to the ring-opening of the thiazine ring of **6** by the nucleophilic attack of **5**, leading to an isomer of the desired final modulator **7** that was very difficult to purge by crystallisation (Scheme 3).

Initially, we ran the reaction very concentrated, and we used a large excess of the chloropropylthiotriazole (**4**) to compensate for the intramolecular cyclisation and to push the chemistry toward the desired bimolecular pathway. We also ended up using a stoichiometric amount of potassium iodide because, even if in theory the halogen exchange should allow using catalytic potassium iodide, preliminary experiments proved that it had to be equimolar to the chloropropylthiotriazole derivative **4** to maximise the yield. As a matter of fact, an experiment run in the absence of potassium iodide gave a yield as low as 76% molar (versus a typical 86−88% molar).

The intention of the proposed work was to adapt the concept of "Design Space" to create a zone of reliable robustness for a process as well as indicating new areas for future operation. Thus, we felt the pivotal C−N bond-formation step deserved a further process understanding effort to optimise the chemistry and enhance its robustness before scaling up. To reach this goal, meeting stringent project timelines, we applied simultaneously a classical Design of Experiment (DoE) approach (a first-order fractional factorial design followed by a robustness study) and a kinetic approach, with the aim to combine the DoE output and the descriptive kinetic model of the chemistry in order to expand the knowledge space of the named reaction.

Design of Experiment Approach. The objectives of this piece of work were to maximise the solution yield and purity of the final compound 1,2,4-triazolyl azabicyclo[3.1.0]hexane (**1**), maximise the selectivity, and minimise the potassium iodide and the unreacted chloropropylthiotriazole derivative (**4**) left in solution at the end of the reaction (Table 1). To do so, a

(4) (a) Iwakubo, M.; Takami, A.; Okada, Y.; Kawata, T.; Tagami, Y.; Sato, M.; Sugiyama, T.; Fukushima, K.; Taya, S.; Amano, M.; Kaibuchi, K.; Iijima, H. *Bioorg. Med. Chem. Lett.* **2007**, *15*, 1022–1033. (b) Lavrador, K.; Murphy, B.; Saunders, J.; Struthers, S.; Wang, X.; Williams, J. *J. Med. Chem.* **2004**, *47*, 6864–6874. (c) Kumar, N.; Kaur, K.; Aeron, S.; dharmarajan, S.; Silamkoti, A. D. V.; Metha, A.; Gupta, S.; Chugh, A.; Gupta, J. B.; Salman, M.; Palle, V. P.; Cliffe, I. A. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5256–5260.

Table 1. **Attributes and specification limits**

attributes	specification limits (%)
1,2,4-triazolyl azabicyclo[3.1.0]hexane	>80
aryl azabicyclo[3.1.0]hexane	<20
N-alkylated derivative	<3

Table 2. **Process parameters and ranges**

	ranges (equiv)		
factors	low	mid	high
chloropropylthiotriazole	1.1	1.35	1.6
triethylamine	2.1	2.35	2.6
potassium iodide	0.2	0.9	1.6
temperature	50	60	70

first-order 2^{4-1}_{IV} fractional factorial design with two centre points was conducted using the Reactarray-SK233 parallel equipment. Four parameters were chosen (Table 2) because they were considered to have the most impact on the reaction output, and 10 reactions were carried out on a gram scale using the SK233.[5]

The first-order linear regression models were fitted for each of the three responses. The significant terms identified by the half-normal plots in the experimental design software, Design Expert (DX-7), formed the basis of the estimated regression models (Figures 1, 2, and 3)[6] calculated using logit transformations.

The statistical model showed an adequate fit to the data and no chemically relevant curvature in all the responses. It also demonstrated that the initial conditions, corresponding to the column "high" of Table 2, could not be significantly improved in the investigated area. Thus, they were selected to further investigate the robustness of the reaction in the proximity of the chosen conditions, bearing in mind some important observations drawn from the DoE study: a high level of KI and chloropropylthiotriazole (**4**) were required to maximise conver-

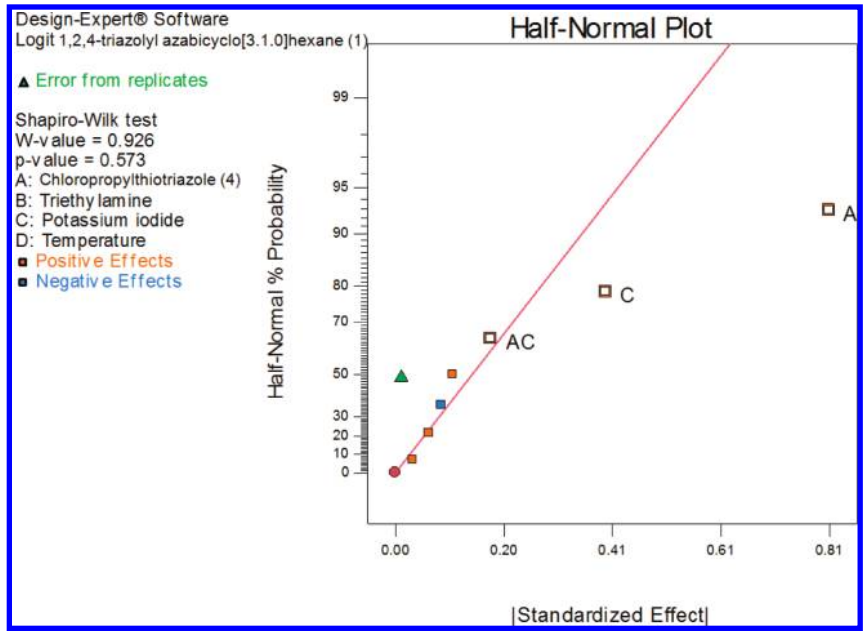


Figure 1. **Half-normal plot for the 1,2,4-triazolyl azabicyclo[3.1.0]hexane yield (1).**

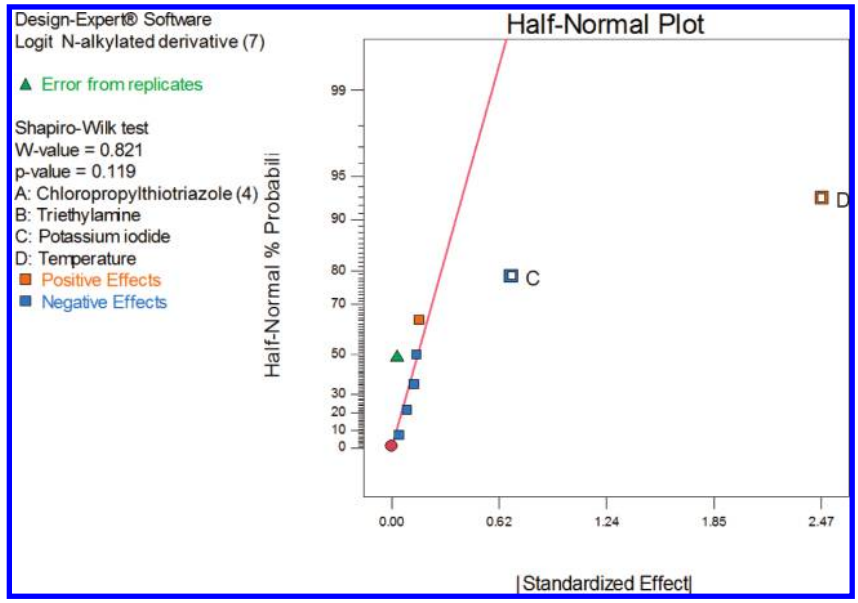


Figure 2. **Half-normal plot for the selectivity (yield of 7).**

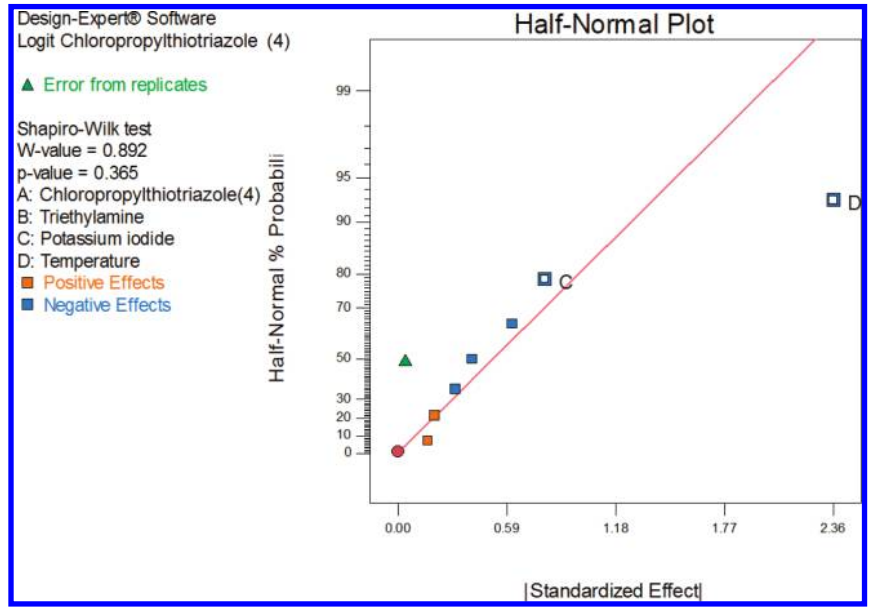


Figure 3. **Half-normal plot for the unreacted chloropropylthiotriazole (4).**

Table 3. **Ranges evaluated in the DoE study**

		factor screening		robustness	
		range evaluated		range evaluated	
factor	current process	low	high	low	high
chloropropylthiotriazole (**4**) (equiv):	1.6	1.1	1.6	1.5	1.7
TEA (equiv):	2.6	2.1	2.6	2.3	2.9
KI (equiv):	1.6	0.2	1.6	1.5	1.7
temperature (°C)	70	50	70	65	75
DMSO (mL/g)	4	4	4	3.6	4.4

sion (Figure 1); the selectivity was increased by a high level of KI and a lower temperature even if the latter leaves behind unreacted chloropropylthiotriazole (**4**) (Figure 2); the temperature rise strongly impacted the chloropropylthiotriazole (**4**) degradation into **6**. A 2^{5-2}_{III} fractional factorial design was carried out by adding the DMSO volume as a further factor. In fact, by running the chemistry on-scale, we realised that the reaction mixture was quite thick, and we wanted to encompass a possible stirrability issue. The ranges evaluated are shown in Table 3.

Unfortunately, the responses' variability was higher than expected (Figure 4), demonstrating that the reaction conditions, despite being the best in the investigated chemical space, were lacking robustness, and in the end, they could have led to a considerable drop in the process yield. In fact, it is clear from Figure 4 that a combination of high temperature and a low amount of chloropropylthiotriazole derivative **4** was detrimental for the 1,2,4-triazol-3-yl-azabicyclo[3.1.0]hexanes (**1**) conversion.

This observation highlighted a common misunderstanding. It is known that the simplest approach to a multiple-response surface robustness is the "overlapping mean response" (OMR) method, in which overlapping response surfaces are used to ascertain a "sweet region", where the three mean response surfaces possess a region of overlap with a desirable multiple-

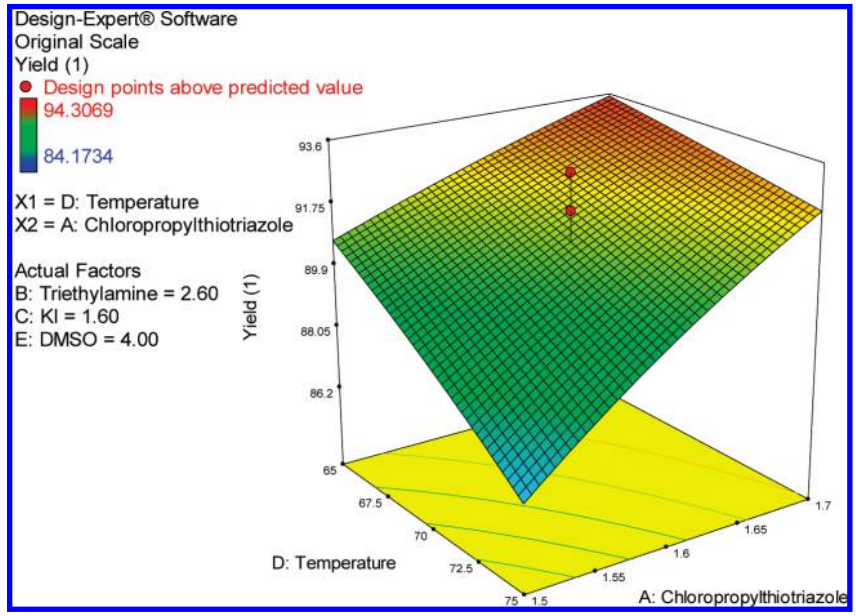


Figure 4. **Dependency of yield (1) from temperature and chloropropylthiotriazole (4).**

Scheme 4. **Synthetic transformations occurring in the reaction mixture**

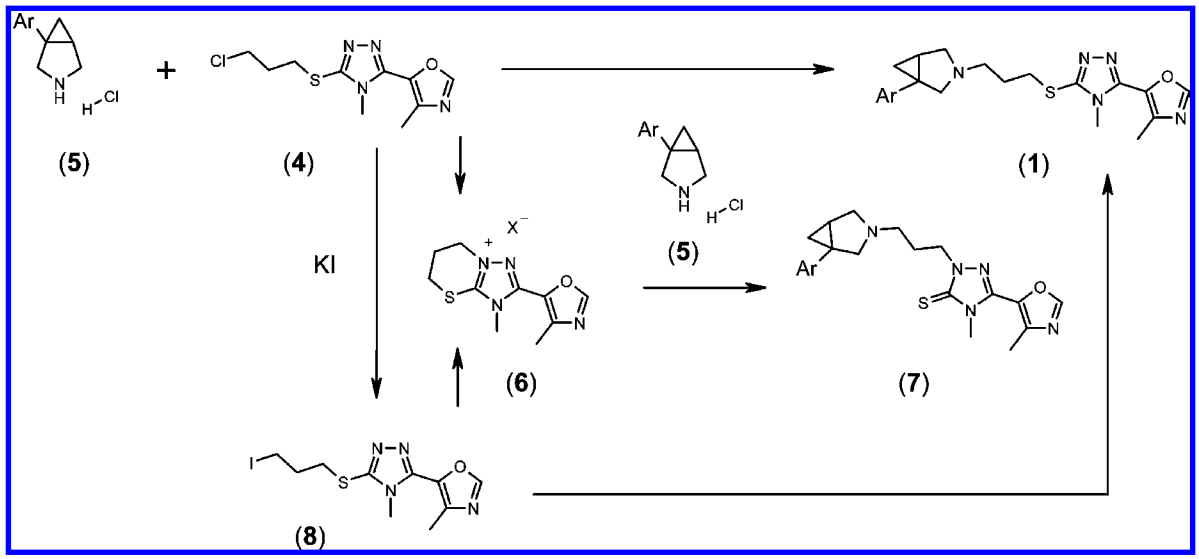


Table 4. **Kinetics model**[16]

reaction	$k>$	E_a	K_{eq}
TEA + H → TEAH	1.00×10^3	not fitted	N/A
protonated azabicyclo[3.1.0]hexane (**5**) + TEA = azabicyclo[3.1.0]hexane (**5**) + TEAH	1.00	not fitted	1.00
protonated 1,2,4-triazolylazabicyclo[3.1.0]hexane (**1**) + TEA = 1,2,4-triazolylazabicyclo[3.1.0]hexane (**1**) + TEAH	1.87	not fitted	1000.00
azabicyclo[3.1.0]hexane (**5**) + chloropropylthiotriazole (**4**) → 1,2,4-triazolylazabicyclo[3.1.0]hexane (**1**) + H + chloride	4.38×10^{-4}	68.14	N/A
chloropropylthiotriazole (**4**) → cyclised derivative (**6**) + chloride	4.62×10^{-5}	94.87	N/A
cyclised derivative (**6**) + azabicyclo[3.1.0]hexane (**5**) → *N*-alkylated derivative (**7**) + H	1.64×10^{-4}	18.43	N/A
chloropropylthiotriazole (**4**) + iodide = iodopropylthiotriazole (**8**) + chloride	3.00×10^{-7}	70	0.02
azabicyclo[3.1.0]hexane (**5**) + iodopropylthiotriazole (**8**) > 1,2,4-triazolylazabicyclo[3.1.0]hexane (**1**) + iodide + H	2.00×10^{-1}	not fitted	N/A
iodopropylthiotriazole (**8**) > cyclised derivative (**6**) + iodide	8.00×10^{-3}	20.00	N/A

response configuration as *per* Table 2. However, this region is often mistaken to be an area where high confidence can be assumed throughout, whereas in reality there is only a chance of meeting requirements, e.g. 50% chance at the boundary of a single attribute.

In addition to OMR plots, other "desirability functions" in Design Expert[7] have been used in a similar context. These functions map the mean responses onto a scalar desirability function which is typically the geometric mean of individual desirability functions. Both approaches have been shown to have serious flaws. First, they do not account for the model parameter uncertainty, which can be substantial.[8] Second, they ignore the correlation structure of the regression model residuals, which can have serious consequences.[9]

In conclusion, the DoE study warned us that the current reaction conditions were not robust but it did not manage to identify better conditions within the investigated area. In a standard DoE approach, at this point, we could have improved our knowledge space only repeating the factor screening in a different chemical region and continuing to fold over until robust reaction conditions would have been identified. However, we decided, from the beginning, that it would have been beneficial to deepen the kinetic pathway[10] of the reaction, capturing the observations that, on first look, could be considered trivial for such a simple C−N bond formation. The idea was then to combine those observations with the initial DoE studies acquiring the highest probability to take the best decision on how further optimise the chemistry.

Kinetic Approach. It was immediately realised that several different transformations were occurring in the reaction pot: the expected S_N2 displacement of the chlorine atom by the

(5) In addition to the four chosen factors, two more parameters can be identified: the amount of DMSO and temperature ramp. The first one was fixed at four volumes to maximize the inter-molecular/intra-molecular ratio considering the restriction due to the minimum stirrable volume in SK233. The temperature ramp was judged influent if compared with the typical 18 h reaction time.

(6) The Half-Normal Probability Plot is a graphical tool that helps assess which factors are important and which should not be considered as significant. Such a diagnostic tool that is used to assess whether the coefficient estimates come from a normal distribution with mean = 0 and variance = σ. If it is the case, all the estimations should lie close to a straight line. This means that no coefficient estimation is significantly different from 0. Otherwise, a departure from the straight line might suggest that the coefficient could be different from 0.

(7) Derringer, G.; Suich, R. *J. Qual. Tech.* **1980**, *12*, 214–219.

(8) Hunter, J. S. *J. Qual. Tech.* **1999**, *31*, 54–57.

(9) Peterson, J. J. *J. Biopharm. Stat.* **2008**, *18*, 959–975.

(10) Carlson, R.; Axelsson, A. K.; Nordahl, A.; Barth, T. *J. Chemom.* **1993**, *7*, 341–367.

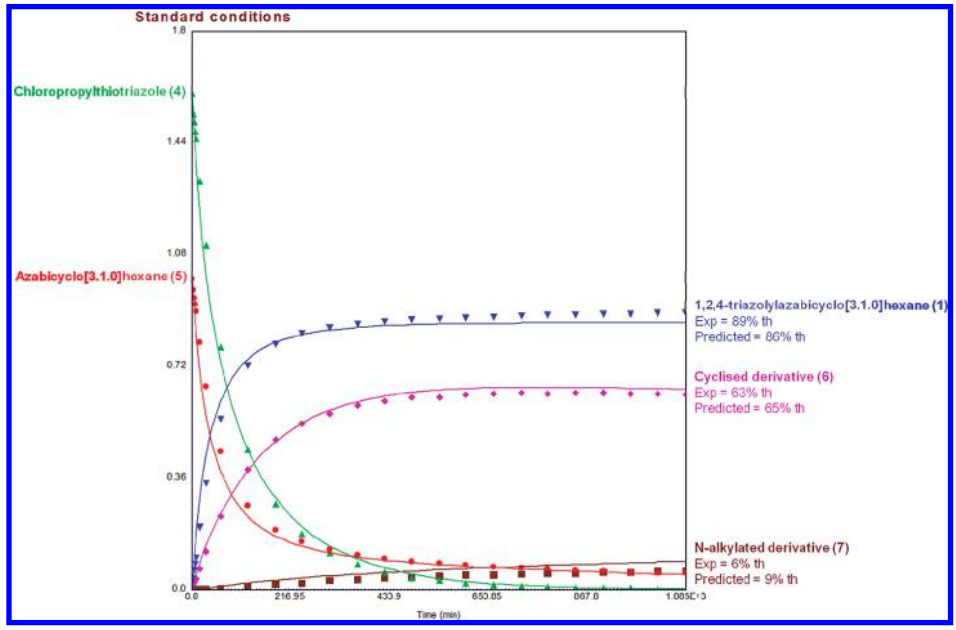


Figure 5. **Overlap of the predictions of the kinetic model and the experimental data for the standard conditions.**

azabicyclo[3.1.0]hexane (**5**), the intramolecular cyclisation of the chloropropylthiotriazole (**4**)[11] and its subsequent reaction with **5** to form the *N*-alkylated derivative **7**, the halogen exchange between chlorine and iodine,[12] and finally the intramolecular cyclisation and S_N2 displacement of the transient iodopropylthiotriazole derivative **8** (Scheme 4). The HPLC was the instrument of choice to monitor the reaction progression, however, because the transient species **8** had never been detected, it was impossible to discriminate between the chlorine-mediated and the iodine-mediated pathway. The issue was overcome by breaking down the chemistry. We ran a first set of reactions (two different temperatures and two different concentrations) in the absence of KI in order to collect accurate data on the chlorine-mediated chemistry. Then, a second set of reactions was run in the presence of KI, where both the pathways were occurring and the data on the iodine-mediated chemistry were obtained by differences with the previous set of reactions. To focus on the halogen exchange, the free base of **5** was used instead of the hydrochloride salt, reducing in this way the equivalents of chloride present in solution. The triethylamine role was clarified by running the displacement in deficit of base. Finally, the importance of the relative ratio of the two starting materials, **4** and **5**, was deepened by running an experiment in deficit of chloropropylthiotriazole (**4**).

The data required for the construction of the kinetic model were collected using the Argonaut Advantage Series workstation,[13] where it is possible to run simultaneously a maximum of four reactions, up to a scale of 250 mL, while collecting the reaction time profiles, in the presence of internal standards, with an online HPLC. The data were fitted using DynoChem[14] software and constrained by some approximations, although minor.[15] The output was a kinetic model able to mimic the behaviour of the two starting materials **4** and **5**, the product **1**, and the two major byproducts **6** and **7** within a 3% molar error (Figure 5). This model was also validated using a completely independent set of data coming from ten runs of the DoE robustness study, achieving the accurate description of the reaction profile and a satisfying maximum error, in the 1,2,4-triazolyl azabicyclo[3.1.0]hexane yield, of 7% molar. The kinetic model (Table 4) provided the following important information on the intrinsic nature of the process: the halogen exchange was the rate-determining step, and the equilibrium lies on the chloropropylthiotriazole (**4**) side. Computer simulations proved that the chemistry needed at least 0.8 equiv of KI to move the equilibrium forward, but its beneficial effect was less pronounced after 0.8 equiv.

Table 5. **Ranges evaluated in the Central Composite Design study**

factor	current process	range evaluated	
		low	high
chloropropylthiotriazole (**4**) (equiv)	1.6	1.0	2.2
TEA (equiv)	3.5	2.0	5.0
KI (equiv)	1.3	0.6	2.0
DMSO (mL/g)	4.0	2.5	5.5
temperature (°C)	65	50	80
temperature ramp (h)	3	1	5

(11) (a) Kanemasa, S.; Nakamura, S.; Kajigaeshi, S. *Chem. Lett.* **1980**, 947–950. (b) Barton, D. H. R.; Bushmann, E.; Hauesler, J.; Holzapfel, C. W.; Sheradksy, T.; Taylor, D. A. *J. Chem. Soc., Perkin Trans. 1* **1977**, *9*, 1107–1114.

(12) (a) Gonzales-Rosende, M. E.; Olivar, T.; Castillo, E.; Sepulveda-Arques, J. *J. Arch. Pharm. Chem. Life Sci.* **2008**, *341*, 690–695. (b) Guliyev, R.; Buyukcakir, O.; Sozmen, F.; Bozdemir, O. A. *Tetrahedron Lett.* **2009**, *50*, 5139–5141. (c) Tu, C.; Osborne, E. A.; Louie, A. Y. *Tetrahedron* **2009**, *65*, 1241–1246. (d) Vasse, J. L.; Levacher, V.; Bourguignon, J.; Dupas, G. *Tetrahedron* **2003**, *59*, 4911–4921.

(13) The Advantage Series 3400 process chemistry workstation provided by Argonaut, is a computer-controlled, automated multi-reactor system designed to offer an increased amount of information in respect to standard manual chemistry. Process parameters can be measured, monitored, controlled, and recorded making use of basic reactor automation.

(14) DynoChem is a set of software tools for process design, characterisation, optimisation and scale-up based on first principles of chemical engineering and physical organic chemistry. The software contains tools designed for the pharmaceutical chemists and engineers, and it is accessible through a Microsoft Excel interface. For further information visit http://www.scale-up.com.

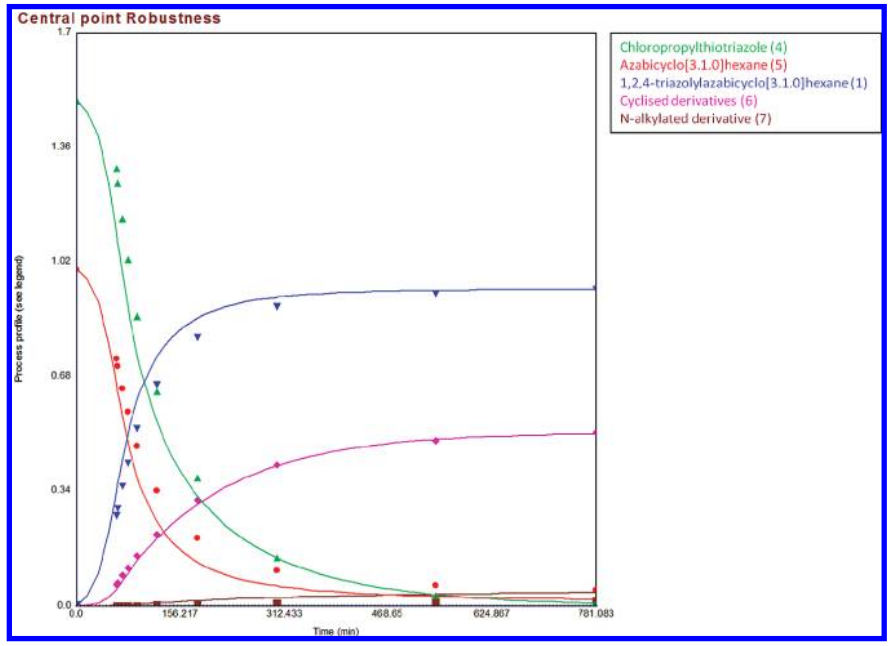


Figure 6. **Central point of the Robustness study.**

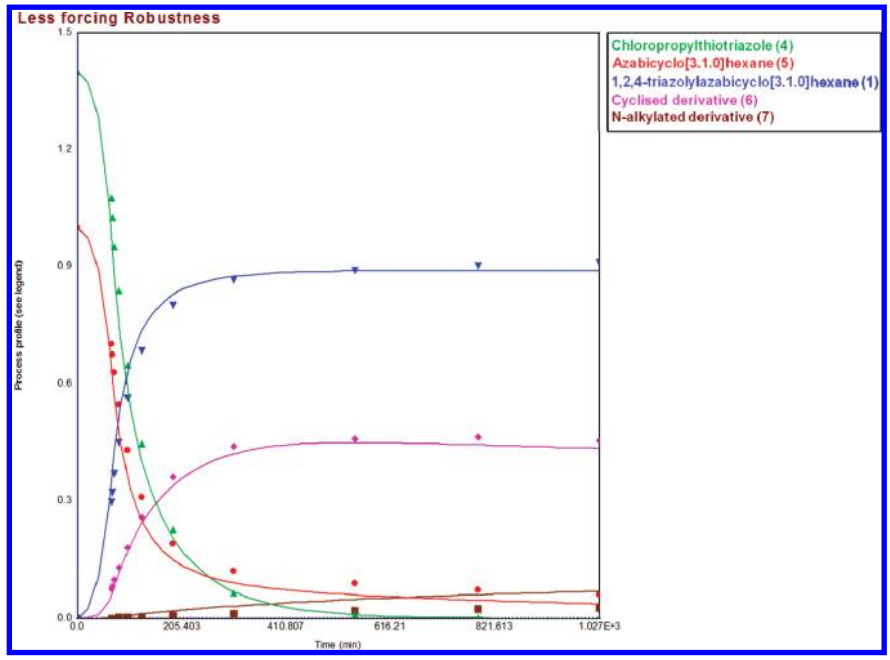


Figure 7. **Less forcing conditions of the Robustness study.**

The TEA pK_A is very similar to that of azabicyclo[3.1.0]-hexane (**5**), and they are a 1000 times more basic than the 1,2,4-triazolyl azabicyclo[3.1.0]hexane (**1**); as a result, a large excess of TEA became important at the end of the reaction when the HCl produced had the tendency to protonate the azabicyclo-[3.1.0]hexane (**5**), slowing down the chemistry. Thus, the increase of the amount of TEA will increase the amount of the free base of **5**, helping the chemistry to proceed. Alternatively, it could be thought to use directly the free base of **5** before running the S_N2 displacement, which would, anyway, slightly complicate the process by adding the extra step required to make such a free base available from its HCl salt. The amount of **4** cannot be reduced sensibly without impacting dramatically the yield. Finally, as observed in the DoE study, the selectivity improved at lower temperature and higher concentration.

We also ran simulations of some specific reaction conditions outside the design space previously investigated by the DoE study (in a classic one factor at a time fashion), and indeed, the easiest way to improve the reaction conversion was to double the amount of TEA (up to 5 equiv). DynoChem predicted a 9% enhancement of the solution yield, and when we ran the

(15) To build the kinetic model, we had to focus on the compounds that had been identified and characterised: starting materials **4** and **5**, product **1**, and main impurities **6** and **7**. The reaction volume was calculated, assuming a density = 1 mg/mL. The response factor of **7** was not measured because an analytical marker was not available; however, considering that **7** is a strictly related isomer of **1**, we assumed it had the same response factor as the main product. Finally we observed a 5% mass imbalance, but we did not impose normalisation by attributing the missing mass to **7**, that in this way becomes a general indicator of the purity of the reaction.

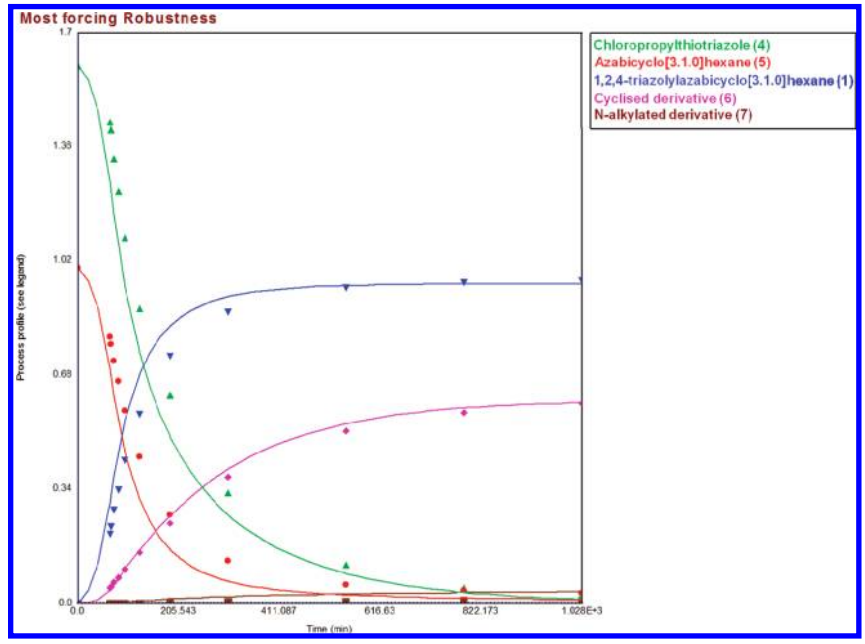


Figure 8. **Most forcing conditions of the Robustness study.**

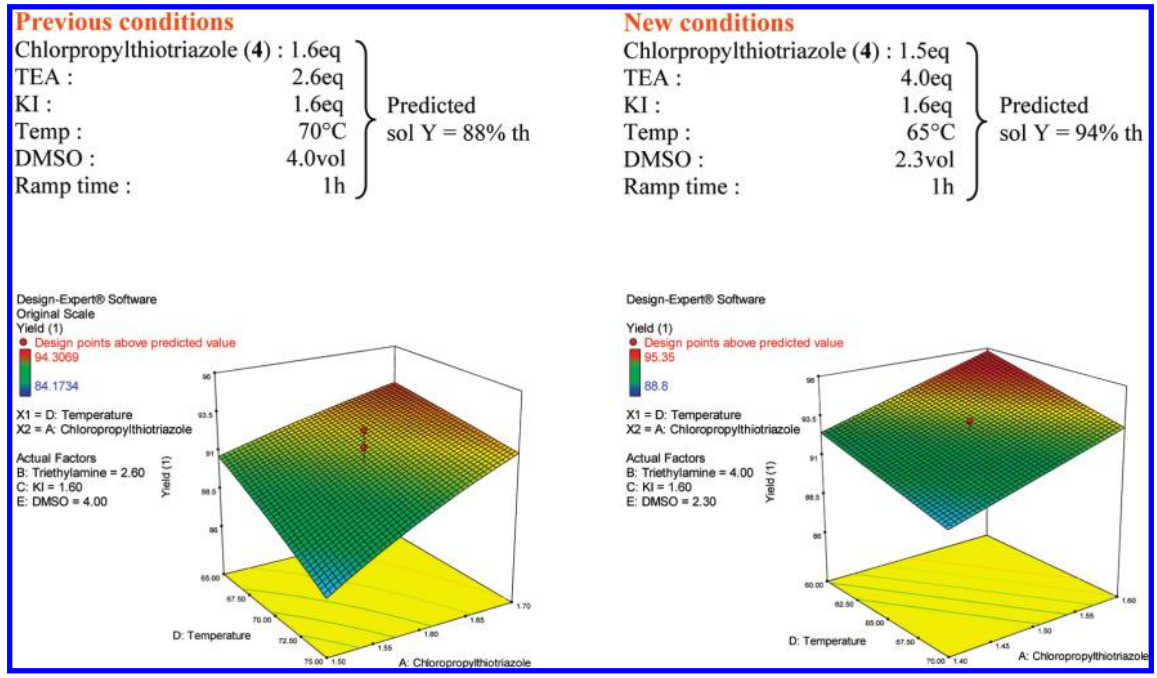


Figure 9. **Comparison of the old and new reaction conditions.**

experiment in the laboratory, we found good agreement (within 1% molar) between the simulation data and the experimental data.

(16) The data relative to the chlorine-mediated pathway resulted in being more accurate because they were directly measured. The equilibrium constant of the halogen exchange was imposed on the base of in-house knowledge. The data relative to the iodine-mediated pathway were less accurate because the input data were obtained by finding the differences between those of the chlorine-mediated pathway. For this reason the absolute value of the kinetic constant and activation energy of the iodine-mediated chemistry was considered as "soft data", whereas the ratio between the two was still valid, i.e. the activation energy of the cyclisation of **8** was 20 KJ/mol higher that the activation energy of the reaction of **8** with **5** to give **1**, whatever its absolute value was. This lack of accuracy on the rate constant and activation energy of the iodine-mediated chemistry didn't affect the overall output of the kinetics model because these two steps were not rate determining, they were the fastest of the entire set.

At that moment, we realized that the mechanism of this simple C−N bond formation was much clearer and we had a way forward to progress the routine synthesis of the novel series of 1,2,4-triazol-3-yl-azabicyclo[3.1.0]hexanes. However, we felt we were not doing as well in quantifying the reliability and robustness of such a step. The DoE studies gave us an idea of the risks, but it did not manage to identify better conditions; whereas the kinetic model had provided higher-yielding conditions, but it did not tell us anything about the robustness of these tested conditions.

Notably, we ran the two approaches simultaneously with the intent to combine the results of the two studies and enlarge the knowledge space of our chemistry. As a matter of fact, each technique had produced half of the required information.

Therefore, it appeared obvious to us to take a final step and try to use both techniques synergistically.

Synergistic Kinetic and Design of Experiments Approach. The idea was to use the DX7 software to get a visualisation of the kinetic model to help identify the significant parameters and their correlations. We set up a completely new factor screening using a full Central Composite Design study, comprehensive of six factors (Table 5), in a wider space than the initial factor screening. The reason was because the kinetic model already proved that higher-yielding reaction conditions were placed outside the area investigated with the initial DoE study. DX7 identified 77 reactions that were simulated using DynoChem and iteratively fed back into DX7.

The selection of an onerous Central Composite Design study was driven by the high amount of retrievable information for such a study at the price of a very short time (few hours) required by the computer simulation.

The results were descriptive of the kinetic model (Pred-R^2 > 0.91 at all responses) and allowed us to identify an area within the investigated space where the predicted yield was higher than 90% molar with a very low level for both starting materials and impurities. The selection of "a point" in the centre of this region provided new improved conditions, whose robustness has been tested simulating a 2^{5-2}_{III} fractional factorial design (Table 5). The DynoChem simulation and the review of the outcome of the 77 reactions of the Central Composite Design study and of the 9 reactions of the robustness was an extremely quick process. We ended up with a possible 94% molar yield versus the initial 88%, and the chemistry was placed in a much more robust area. To complete this piece of work, we ran in the Argonaut workstation only three confirmatory experiments of the 86 reactions we had simulated, (1) the new improved reaction conditions (Figure 6), (2) the forcing (Figure 7), and(3) the mildest conditions (Figure 8) of the Robustness study. We observed a good agreement between the DynoChem description and the experimental data, proving that we had identified new higher-yielding reaction conditions in the investigated chemical space. The improvement concerned not only an increase in the solution yield of 6% molar but, more importantly, confirmed the identification of much more robust reaction conditions (Figure 9, right). The space investigated during the last Robustness study was an area where "the multidimensional combination and interaction of input variables and process parameters (i.e. the factor investigated during the study) have been demonstrated to provide assurance of quality", and it was therefore, according to the ICH Q8 Guidance, the Design Space of the reaction (see Table 6).

Table 6. **Reaction Design Space or ranges evaluated in the final Robustness study**

factor	current process	range evaluated	
		low	high
chloropropylthiotriazole (**4**) (equiv)	1.5	1.4	1.6
TEA (equiv)	4.0	3.5	4.5
KI (equiv)	1.6	1.5	1.7
DMSO (mL/g)	2.3	2.0	2.6
temperature (°C)	65	60	70

Conclusion

We have reported how we managed to deliver the Design Space of the key S_N2 displacement step between the 1,2,4-triazol-3-yl-halide derivative and variously substituted azabicyclo[3.1.0]hexanes for the reliable synthesis of a novel class of selective dopamine (DA) D_3 receptor antagonists. In doing so we exploited the capabilities of the two individual techniques, DoE and kinetics, identifying their limits and using them synergistically to maximise the throughput.

Acknowledgment

We acknowledge Paolo Repeto and Mohammad Yahyah for the invaluable advice and encouragement they have provided us during the preparation of this paper.

Supporting Information Available

The kinetics model and the data required for its construction, data of the three validation experiments of the virtual robustness study. This material is available free of charge via the Internet at http://pubs.acs.org.

Received for review June 24, 2010.

OP100176U