Flow assisted synthesis of SR 142948A a Neurotensin probe

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Abstract: Abstract Text, 800-1000 characters.

Introduction

Neurotensin (NT, **1**) is an endogenously expressed tridecapeptide first isolated from bovine hypothalamii in 1973.[1] NT plays many important roles in a variety of biological processes including temperature control,[2] pain sensation,[3] modulation of appetite[4] and pituitary hormone secretion.[5] As a modulator of the bodies dopaminergic systems, disruption of NT's binding has also been proposed as a possible treatment of schizophrenia and Parkinson's disease.[6] In addition, up regulation of NT receptor (NTR) expression has been observed in various cancers of the Lung,[7] Breast,[8] Pancreas,[9] Pituitary[10] and of the Prostate and therefore investigation of is physiological mechanisms is an important area of study.[11] Unfortunately, as a potential therapeutic and investigative tool NT itself has several drawbacks. As a oligopeptide, NT has inherent poor stability *in vivo* and is degraded by several common endopeptidases and metalloproteases.[12] Its size also prevents easy passage across the blood brain barrier resulting in poor bioavailability requiring injection of NT directly into the CNS[13] and finally, NT is only capable of NTR agonism. Consequently synthetically viable, easily modified small molecule probes capable of tailored interaction with the NTR (agonist/antagonist) represent powerful investigatory tools in medicinal chemistry.

As part of our synthetic efforts to prepare a tool box of NT molecular probes we had already explored the preparation of Meclinertant (**2**), a structurally related NT modulator using a flow chemistry approach.[14] In an extension of this work we herein report on a more synthetically challenging derivative SR 142948A (**3**) which required a range of new chemistries to be invented and translated to flow.[15] We envisaged a convenient convergent synthesis allowing assembly of this new derivative **3** from three fragments, two of which were common to the previous Meclinertant synthesis, and had already been prepared at scale.[16] Here we report on selected flow methods achieving the synthesis of compound **6**. The general retrosynthetic disconnection of the new key fragment **6** is shown in figure 2, which would ideally be accessed in a multi-step synthesis from the commercial and readily available starting material 2-Isopropyl aniline (**10**).

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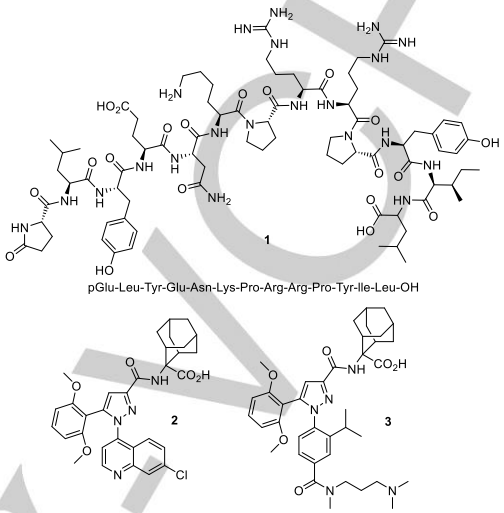


Figure 1. Structures of Neurotensin, *Meclinertant,* and SR 142948A.

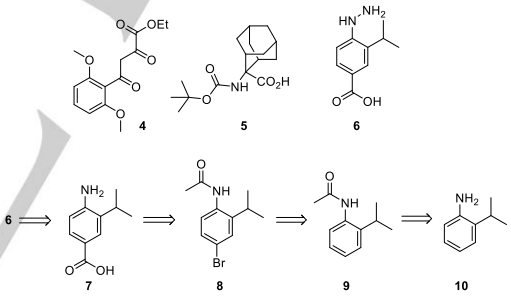


Figure 2. Synthetic fragments of SR 142948A and proposed retrosynthesis of hydrazine (**5**).

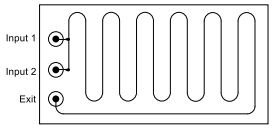
Results and Discussion

We commenced our synthesis by first targeting the carboxylic hydrazine derivative **6**. Our approach was to perform an acylation on the isopropyl aniline (**10**), necessary to regulate the subsequent bromination step and furnish selectively intermediate **7**. This would then enable either a cyanation or carbonylation followed by hydrolysis to access the desired carboxylic acid **9**. Diazotisation and direct reduction would yield the corresponding hydrazine product **6**. Although encompassing conceptionally straightforward chemistry many of these transformations have intrinsic risks associated, especially when conducted at scale such as exotherms (acylation), or issues due to hazardous (diazotisation) or toxic (carbonylation, cyanation) reagents/intermediates. It was anticipated that through the

adoption of flow processing techniques these risks could be managed, minimized or completely avoided.

The initial acyl protection (**10**→**9**) was associated with a large exotherm when dichloroethane solutions of acetic anhydride (1.73 M; 3 mL/min; 1.1 equiv.) and aniline **10** (1.57 M; 3 mL/min) were combined (Scheme 1). The process required active cooling to prevent runaway in batch but could be easily performed in a microfludic mixing chip as a flow process, which ensured good thermal transfer to the surrounding environment. In this way, the solution could be continuously processed maintaining a temperature <55 °C. Beneficially it was discovered that the following bromination step required heating to achieve complete conversion and that by creating a sandwich assembly from two additional mixing chips (inputs for the *N*-bromosuccinimide) in direct thermal contact with the first acylation reactor the excess heat produced could be used in a productive fashion to warm the reaction media for the second step (Figure 3).[17]

a)



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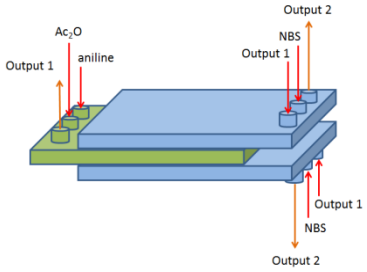
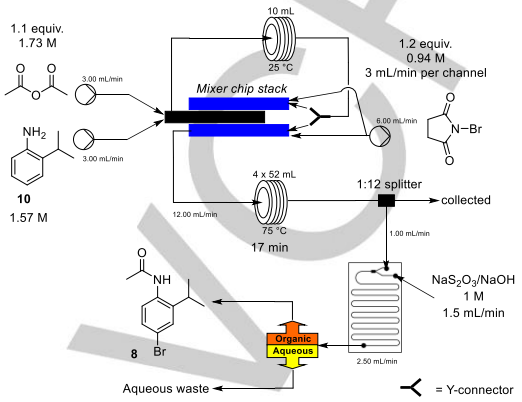


Figure 3. a) single layer microfluidic chip. b) sandwich chip device for thermal transfer between reaction steps.

Therefore the reaction stream from the first acylation step was split and directly combined with input streams containing NBS in dichloroethane (DCE) (0.94 M; 3 mL/min in two parallel channels; 1.2 equiv.) (Scheme 1). The two flow streams were reunited and then passed through a further residence time coil (4 x 52 mL) maintained at 70 °C to ensure complete bromination. The reaction mixture was then quenched and an in-line work-up was performed by mixing with a stream of basic sodium sulphite and separated using a mixer-settler membrane unit. The device was constructed around a Biotage universal separator[18] which allowed the bifurcation of the organic product containing phase and the aqueous waste (Figure 4).[19] Run as a continuous process this allowed the isolation of the amide product **8** by direct evaporation. In total over 120 L of organic solution was processed over the course of 167 h (~ seven days) generating an isolated mass of 11.52 kg of product equating to 96% yield.

Furthermore, we were able to generate a refilling schedule for the input stock solutions (2.5-5 L batches) based upon their consumption flow rates that enabled the efficient recycling of recovered DCE from the final evaporation stage. Consequently the process only required the use of 26.7 L of DCE amounting to a significant reduction in the amount of organic solvent used.



Scheme 1. Flow reactor schematic for the synthesis of amide **8**.

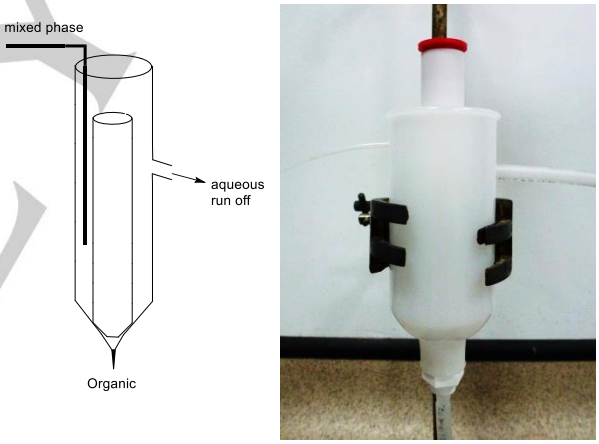
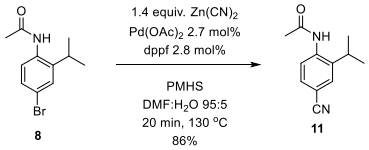


Figure 4. In-line extractor for organic-aqueous separation.

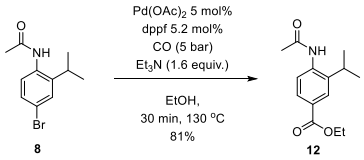
With kilogram quantities of the bromide **8** in hand, further derivatization to the corresponding nitrile **11** was undertaken (Scheme 2). Experiments were initially evaluated using an automated microwave setup. Initial investigation of reaction conditions showed the importance of including a reductant in the reaction mixture (Table 1: entries 1-4). In the absence of polymethylhydrosilane (PMHS)[20] no conversion was observed with either supported or solution phase catalysis. Screens of common supported forms of palladium (Table 1; entries 5, 6, 8, and 9) showed lower conversion and extended reaction times compared to the use of solution phase palladium(II) acetate. A solvent screen determined a mixture of DMF/H_2O (95/5) gave optimal results.



Scheme 2. Optimised batch conditions for synthesis of nitrile **11**.

To process material on a semi-preparative scale, the automated microwave reactor was utilized as a sequential batch process reacting 39 mmol of substrate and allowing 6.8 g of nitrile **11** to be isolated in 86% yield. Although this approach was convenient for small laboratory scale preparation the observed low solubility of the reagents (only becoming soluble during the reaction progression) lead us to conclude that this would be a difficult reaction to translate to continuous flow and therefore alternative approaches where sort.

The possibility of performing a carbonylation reaction was investigated. Again the initial investigation and optimization work was conducted using an automated microwave setup. The use of microwave vials allowed easy introduction of CO gas and pressurization of the reaction mixture up to 5 bar. Both ethanol and methanol could be successfully used in the transformation however ethanol showed the highest conversion whilst minimizing the amount of protodehalogenation (as determined by ^{1}H NMR analysis of the crude reactions).



Scheme 3. Optimised batch conditions for synthesis of ester **12**.

Using the microwave automation a sequential run of 10 reactions allowed the processing of the bromide **8** on a 59 mmol scale generating 12 g of material in 81% isolated yield (Scheme 4). Additionally it was observed that the yield for each run was consistent (indicating catalyst/substrate stability over time) and the reaction mixtures remained essentially homogeneous throughout the processing (some indication of Pd black formation was noted). This immediately allowed us to consider translation of the conditions to a flow based system.

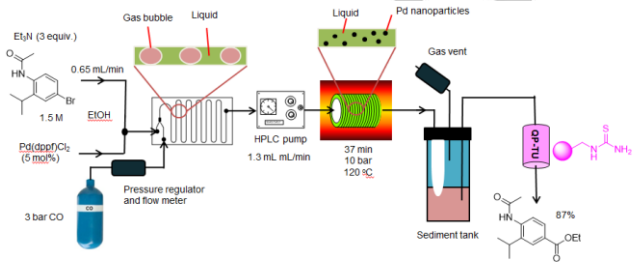
Table 1. Optimisation of microwave cyanation conditions.

Ent.	Catalyst[a]	Temp °C	Additive	Solvent[c]	Time/h	Conv. %
1	$Pd(OAc)_2$	80	dppf/ PMHS	DMF/H_2O	3	66
2	$Pd(OAc)_2$	80	dppf	DMF/H_2O	3	0
3	$Pd(OAc)_2$ EnCat[b]	80	dppf/ PMHS	DMF/H_2O	3	74
4	$Pd(OAc)_2$ EnCat [b]	80	dppf	DMF/H_2O	3	0
5	$Pd(OAc)_2$/dppf EnCat [b]	80	PMHS	DMF/H_2O	3	6
6	Pd-NP EnCat	80	dppf/ PMHS	DMF/H_2O	3	77
7	$Pd(dba)_2$	80	dppf/ PMHS	DMF/H_2O	3	78
8	$Pd(OAc)_2$ Chemdose	80	dppf/ PMHS	DMF/H_2O	3	5
9	$Pd(OAc)_2$ EnCat	80	dppf/ PMHS	DMF/H_2O	3	57
10	$Pd(OAc)_2$	120	dppf/ PMHS	DMF/H_2O	1	>98
11	$Pd(OAc)_2$	120	dppf/ PMHS	$iPrOH/H_2O$	2	73
12	$Pd(OAc)_2$	120	dppf/ PMHS	$MeCN/H_2O$	2	63
13	$Pd(OAc)_2$	120	dppf/ PMHS	1,4-dioxane/H_2O	2	75
14	$Pd(OAc)_2$	120	dppf/ PMHS	H_2O	1	69
15	$Pd(OAc)_2$	120	dppf/ PMHS	DMF	1	87
16	$Pd(OAc)_2$	120	dppf/ PMHS	iPrOH	2	78
17	$Pd(OAc)_2$	120	dppf/ PMHS	1,4-dioxane	2	75
18	$Pd(OAc)_2$	120	Xantphos/ PMHS	DMF/H_2O	1	>98

[a] Standard loading 2.2 mol%. [b] Loading 4.4 mol% based upon Pd loading. [c] solvent ration 95:5

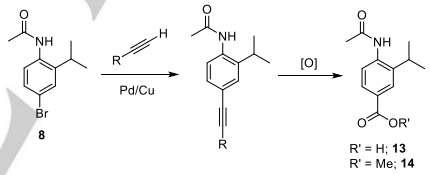
The direct introduction of CO gas at high pressure to perform carbonylation chemistry has been widely demonstrated using several designs including simple tube-in-tube systems.[21] However, although the handling and introduction of high pressure gases to flow systems is achievable the final design of our flow system was restricted to a low pressure CO injection system because of concerns raised by our then current Health and Safety Officer. Despite this we still felt that the system presents certain engineering features that are worth highlighting as they can be easily adopted to solve related issues commonly encountered in other chemistries, for example the introduction of semi-volatile amines.

The configuration of the flow reactor used is depicted in Scheme 4. The system consisted of a mixing chip fed by an ethanolic solution of the substrates, catalyst and base to be blended with a low pressure (3 bar) input of CO regulated via a flow meter.[22] A plug flow regime was generated and used directly as the fed for a HPLC pump.[23] The pump was used to up-regulate the pressure to 10 bar making the reaction mixture homogeneous and delivering sufficient CO for efficient carbonylation in the subsequent coil reactor stage (Polar bear plus[27] ~37 min; 120 °C). It was noted that during the reaction colloidal palladium particles formed in the coil reactor (additives like tetraalkyl ammonium salts, DMF and alternative metal chelating ligands were investigated but failed to have any effect on the process). This gave no issues with reactor blocking through aggregation with the particulates being efficiently progressed through the reactor even over prolonged reaction periods. However, clogging of the inline backpressure regulator (BPR), required to maintain the system pressure, rapidly occurred. In addition, significant and uncontrolled degassing was also an issue at the exit of the reactor; this was made more problematic due to the particulates in the system which where explosively jettisoned from the flow stream leading to etching of the collecting vessel and containment problems. To overcome both of these issues we constructed a simple membrane partitioned sedimentation tank which also acted as a controlled gas exhaust. The unit was constructed from a stainless steel pressure chamber with an embedded thermocouple and pressure dial. An adjustable pressure release valve was fitted into the cap (to enable discharge of the gas). The input feed delivered the solution and particulates from the reactor and the exit line was fitted with a polytetrafluoroethene (PTFE) solvent filter.[26] The output line was connected to an HPLC pump which progressed the filtered product solution. Although this approach worked well over short processing times but we noticed that build-up of the particulate material around the in-line PTFE solvent filter started to impair the pumping efficiency of the exit HPLC pump by restricting its input flow. This was easily observed by noting the decline in the flow rate of the exit solution, indeed, HPLC systems are well known for having very little tolerance on the pressure drop of the input line. We found that by alternatively using a extraction thimble filter[25] fitted at the inlet of the sedimentation chamber and utilizing the pressure drop from the reactor more effective filtration of the particulate matter could be achieved. In this way the pressure feed of the solvent for the exiting HPLC remained constant. In this way the reactor could be run for up to 8 hour before particulate build-up required a scheduled shutdown and exchange of the thimble filter. Finally, the material pumped from the sedimentation tank was directed through a column of QP-TU (a thiourea functionalised resin) to remove residual metal contaminants before batch collection and solvent evaporation. The crude material was directly triturated with a mixture of water:ethanol 20:1 and filtered followed by washing with water. The tan coloured solid was air dried to yield the desired ester product in excellent purity as assessed by ^{1}H NMR and LC-MS. During a typical 8 h run 120 g of starting material could be processed in 87% isolated yield equating to a productivity of 12.7 g/h (58.5 mmol/h).



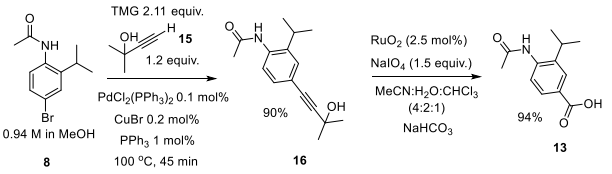
Scheme 4. Flow reactor design for the synthesis of ester **12**.

Although delivering high quality material this approach didn't fulfil our target throughput requirements for a continuous process and was uneconomic due to the high loading and associated cost of the palladium and associated dppf ligand. We therefore sort other approaches, a strategy which offered simplicity and directness seemed to be a Sonogashira coupling followed by an oxidative cleavage of the acetylene to furnish a carboxylic acid moiety (Scheme 5).



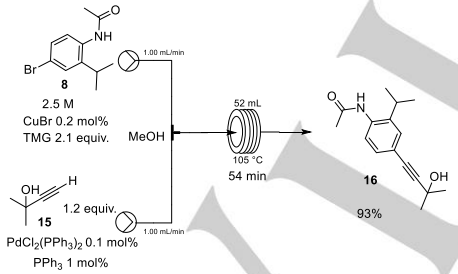
Scheme 5. New strategic approach to the target structures **13** and **14**.

We initially performed a scoping evaluation on the Sonogashira reaction using a series of automated microwave reactions systematically assessing; catalysts (palladium and copper sources), bases, solvents, concentration/stoichiometry, temperature and reaction times. All reactions were rapidly assessed as % conversion using calibrated LC-MS analysis (a summary of the findings appear in the supplementary information). From this study a set of optimum conditions (90% isolated following column chromatography) were defined that seemed to also favour translation of the reaction to a flow processing regime (Scheme 6). We were also able to demonstrate that in the presence of a basic solution of RuO_2 and the co-oxidant KIO_4 (or oxone) oxidative cleavage to the alkyne occurred (3.5 h) to give 94% isolated yield of the corresponding carboxylic acid product **13**.[28]



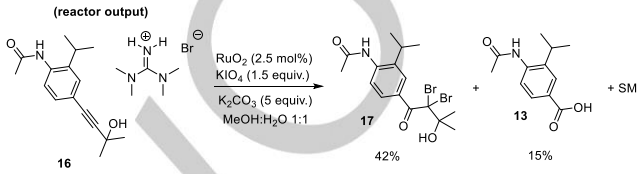
Scheme 6. Optimised conditions for microwave Sonagashira coupling reaction.

Based upon these findings we embarked upon the translation of the Sonagashira step to flow. First however an additional incubation study (48 h) was performed where the different reaction components were tested for their stability, compatibility and prolonged solubility in different combinations (see the SI). From this investigation a simple two channel flow set-up was conceived; Channel A, delivered a flow stream comprising of the aryl bromide (2.5 M), the TMG base and the CuBr in MeOH (Scheme 7). Channel B, a methanolic solution of the propargylic alcohol (3 M), palladium catalyst and associated PPh_3 ligand.[29] The two streams (1:1 flow rate of 1 mL/min) were united at a simple T-piece mixer before progressing into a heated coil reactor (2 conjoined 52 mL Polar Bear plus systems) maintained at 100 °C (a 250 psi BPR was used to maintain system pressure). The exiting solution indicated a steady state conversion of 93% which could be driven to complete conversion by simply raising the reactor temperature to 105 °C. This gave a theoretical throughput of 0.15 M/h or 38.9 g/h. The product could be readily isolated by evaporation of the solvent and pouring the resulting crude oil obtained into a stirred solution of 3 M HCl (0 °C). The product precipitated over the course of ~ 1 h and could be filtered and dried at 40 °C under vacuum to give an overall isolated yield of 93% (5 h run). To demonstrate the robustness of the system a continuous 5 day run was performed collecting the reactor output into individual batches equating to daily production which were then manually worked up. Overall a yield of 90%±3.7 was obtained equating to a total 4.2 Kg of isolated material.



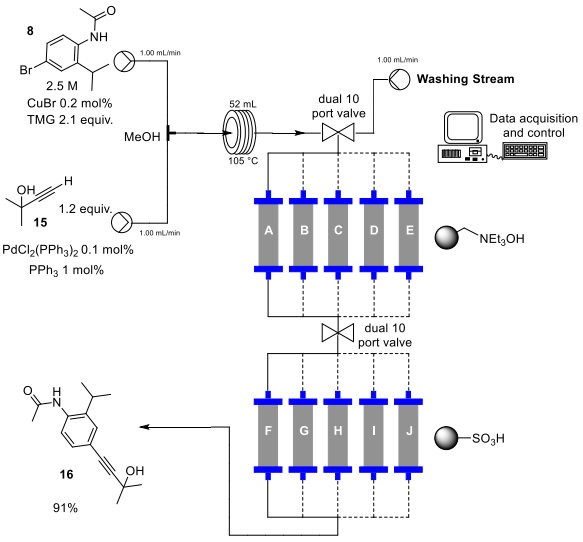
Scheme 7. Flow Sonagashira coupling reactor.

It was our initial intention to attempt direct oxidation of the acetylene product **16** without work-up and isolation. However, we found that attempts to directly use solutions of the reactor output under the previously attempted oxidation conditions (RuO_2/$NaIO_4$) inevitable gave an alternative major di-brominated adduct **17** (Scheme 8). Presumable this product formed through preferential oxidation of bromide (carried TMG.HBr salt) generating *in situ* bromine which adds to the alkyene leading to **17** through hydrolysis. This was partially confirmed by demonstrating that when basic (K_2CO_3) solutions of acetylene **16** in 1:1 solutions of MeOH/H_2O were treated with bromine the identified product **17** rapidly formed. As all related oxidation processes would potentially generate the same difficulties we therefore investigated adding an additional purification stage.



Scheme 8 By-product formation in the oxidation of acetylene **16**.

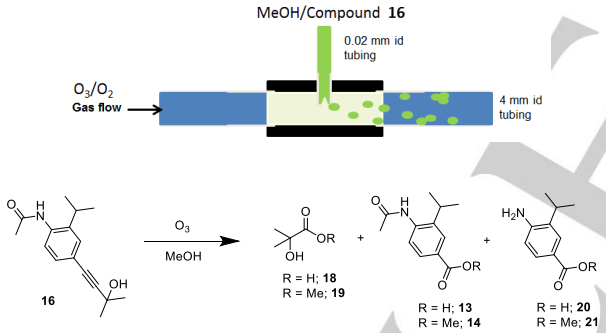
To achieve the reaction stream clean-up we elected to employ a dual stage scavenging process utilising first a cartridge of Ambersep 900 OH resin, to neutralise the TMG.HBr salt, followed by a second cartridge of a sulfonic acid resin (QP-SA) which sequestered the free TMG base. Although a mixed bed of the two resins was also effective we found it much more efficient to stage the scavenging sequence as this helped in constructing a simple automated column exchange process; enabling interchange of the two types of scavenging columns as they became depleted. Introduction of replacement columns and ensuring the washing/regeneration of depleted units was conducted by a simple multi-port valve system as previously described.[30] For this process we set the unit to trigger based upon a timing event to correspond to the sequestering threshold of the various packed columns as calculated from the flow rate and theoretical concentration of the TMG input. This generated a flow stage as depicted in Scheme 9.



Scheme 9. Flow process for the synthesis of acetylene **16**.

Although this inline work-up was effective we were unable to achieve an efficient oxidative cleavage of the alkyene (<42% conversion) without first effecting a solvent swap to the original acetonitrile/water/$CHCl_3$ mixture. As we desired to produce a telescoped sequence linking the oxidation to the Sonagashira step we decided to explore more amenable chemistries.

There are only limited studies in the literature regarding the ozonolysis of alkynes.[31] In addition there is no clear view as to which substrates yield anhydride or carboxylic acid products valuable for our study and which substrates result in benzil derivatives which would be of less use.[32] We however thought it would be of speculative interest to evaluate the transformation with regard to our particular substrate **16**. We aimed to make use of the fact the ozonolysis reaction could be performed in an alcoholic solvent to, in theory, aid the breakdown of the ozonide intermediates and fragment the intermediate anhydrides if produced. Consequently we wished to perform the ozonolysis directly on the methanolic reactor output used in the preparation of **14** (Scheme 5). Several flow reactors have been reported for ozonolysis reactions[35] however we elected to use a simple wavy annular flow[33] ozonolysis design[16b] which we had previously employed successfully at scale (Scheme 10). This used a T-assembly to inject a flow of the substrate **16** from a capillary input into a fast flowing gas flow of the O_3.



Scheme 10. Flow ozonolysis of acetylene **16**.

From the initial ozonolysis runs we were pleased to identify the oxidative fragmentation products **13**, **14**, **21**, **22** and 2-hydroxy-2-methyl-propanoic acid **18** as well as the corresponding methyl ester adduct **19**. Table 2 summarizes some of the further informative optimisation results. As can be seen temperature controls the distribution of aryl products through direct and secondary hydrolysis however it has a nominal influence on conversion. Concentration and residence time were found to be the main parameters determining starting material consumption. The addition of additives such as acids (e.g. HCl, PTSA) or bases (pyridine, DMAP, K_2CO_3) neither enhance conversion or reaction rates and had only small effects on product proportions.

For the final flow process we elected to use a concentration of 1 M and flow rates of 2 and 0.85 mL/min for oxygen and substrate streams respectively at ambient temperate giving quantitative conversion. This produced a good match for the concentration of the exiting stream from the Sonagashira step. In addition although the oxidation of alkynes to anhydrides avoids the production of classical intermediate ozonides and peroxides upon analysis the processed solution contained low level peroxy species (12 mg/L).[35] To aid decomposition of these residual peroxy species a short packed column of MnO_2 was added which reduced the peroxy components below detectable levels. A processing capacity of 51 mmol/h was achieved which would equate to approximately 12 g/h of product. Figure 4 shows ^{1}H NMR spectra of the crude reaction mixture directly following evaporation of the output stream. The final product could be isolated by solvent evaporation and allowing the crude to crystallise upon standing in a 65% yield after filtration and washing with cold hexane. The corresponding carboxylic acid **20** could be obtained quantitatively by heating the same crude material dissolved in 3 M HCl at 125 °C for 30 min by passage through a tubular flow coil reactor. As these acidic conditions met our need for the subsequent sequence we anticipated using this solution directly in the next operation.

Table 2. Subset optimisation of flow ozonolysis reaction conditions.

Ent.	Oxygen flow rate[a]	Substrate flow rate[b]	Temp. °C	Conv. %	Ratio[f] **13**:**14**:**20**:**21**
1	1.0	1.0	0	100	15:79:2:4
2	1.5	1.5	0	90	13:83:2:2
3	2.0	1.5	0	100	11:86:1:2
4	3.0	2.0	0	86	10:88:1:1
5	4.0	2.0	0	74	9:89:1:1
6	2.0	1.5	-78	100	2:98:0:0
7	2.0	1.5	rt	100	19:73:3:5
8	2.0	1.5[c]	rt	97	18:72:4:6
9	2.0	1.5[d]	rt	73	Nd
10	2.0	1.5[e]	rt	64	Nd
11	2.0	1.25[e]	rt	82	Nd
12	2.0	1.0[e]	rt	96	18:74:2:6
13	2.0	0.75[e]	rt	100	19:76:3:5
14	2.0	0.85[e]	rt	100	18:76:3:3
15	2.0	0.95[e]	rt	96	17:76:3:4

[a] O_2 flow rate was controlled using a Brukhurst flow meter [b] 0.5 M in MeOH. [c] 0.7 M in MeOH. [d] 0.9 M in MeOH. [e] 1 M in MeOH. [f] As determined by UV-LC rounded to nearest %. Nd = not determined.

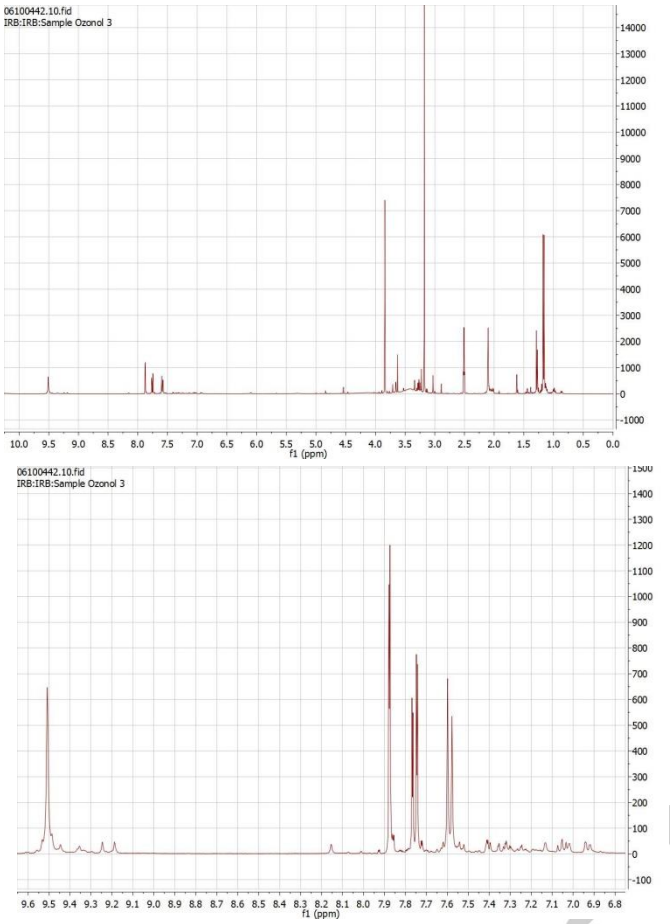
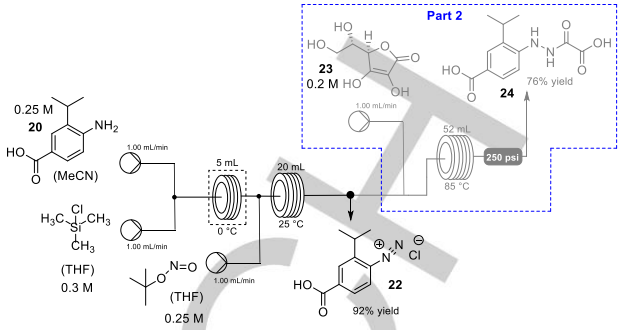


Figure 4. a) 1H NMR of the crude reaction from Ozonolysis – entry 2 Table 1 and b) the expansion of the aromatic region of the same spectra.

To access hydrazine **6** required a further two step sequence of diazatisation and reduction. Here we were able to rely on some previously devised flow chemistry involving reaction of a diazonium salt generated *in situ*[36] with ascorbic acid to affect the reduction (Scheme 13).[37] As an alternative and backup we also evaluated a more classical reduction involving a mixture of $SnCl_2$ and HCl which was shown to work well in batch.

To evaluate the transformation of aniline **20** to its hydrazine derivative **6** we initially started with isolated and purified material (Scheme 11 – initially excluding part 2). A bulk solution of 4-amino-3-isopropylbenzoic acid (**20**) was prepared in acetonitrile and pumped at a flow rate of 1.00 mL/min to combine with second solution of trimethylsilyl chloride in THF also with a flow rate 1.00 mL/min. The combined flow was directed into a short 5 mL FEP (fluorinated ethylene propylene copolymer) reactor coil which was maintained at 0 °C (2.5 min residence time). The exiting stream was further combined with a THF solution of *tert*-butyl nitrite (flow rate 1.00 mL/min) and reacted in a 20 mL FEP coil at rt (6.5 min residence time). A precipitate formed during the reaction which was collected at the exit of the reactor via filtration to give the diazonium salt **22** in an excellent 92% isolated yield.

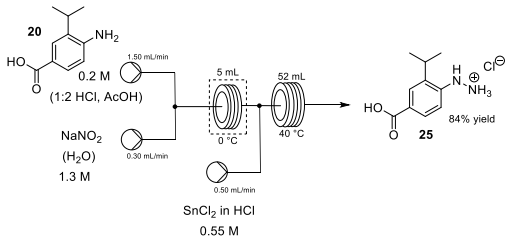


Scheme 11. Flow diazotisation of aniline **20** and part 2 ascorbic acid (**23**) reduction.

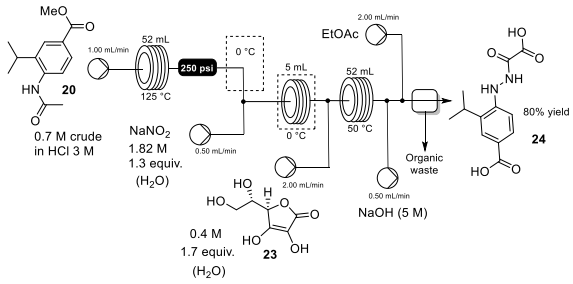
Extending this sequence (Scheme 11 - Reactor part 2) we incorporated a further pump to deliver an aqueous stream of the ascorbic acid (**23**) reductant (flow rate 1.00 mL/min) and passed the flow stream into a coil reactor (52 mL Polar Bear Plus[27], 85 °C - 13 min residence time). A 250 psi BPR was used to maintain the system pressure. During passage through the heated reactor the solution became homogeneous and was collect at the reactor exit. A batch work-up was then used to extract the corresponding oxamic acid **24** to evaluate the effectiveness of the sequence.[39] First the output solution was diluted with ethyl acetate and extracted with 2 M NaOH (pH 10-11). The aqueous extract was separated, re-acidified and back extracted into ethyl acetate. The solid material obtained from evaporation of the solvent was crystallized from a 7:1 mixture of THF:Et_2O to yield the hydrazine adduct **24** in 76% yield as a pale yellow solid.

Having set a bench mark with the ascorbic acid approach we next evaluated the alternative tin mediated process as shown in scheme 12. Again, using a simple three pump system this time based upon a peristaltic delivery system (E-Series Flow reactor) a solution of the aniline **20** in a 1:2 mixture of HCl and AcOH (0.2 M) was mixed with a solution of aqueous $NaNO_2$ (1.3 M) at a T-piece (PTFE - polytetrafluoroethylene). The mixture was then directed through a FEP flow coil reactor maintained at 0 °C (2.8 min residence time). A further flow stream containing the $SnCl_2$ in HCl (0.55 M) was united and the mixture passed into a second heated FEP flow coil (52 ml, 40 °C, Polar Bear Plus[27] – 23 min residence time). A suspension rapidly formed but could be easily propagated through the reactor without causing blockage. The reactor output was dispensed directly onto a sintered filter. After 60 min of operation the filter cake was washed with water giving 3.48 g, 84% isolated yield of the hydrazine salt **25** after drying.

The success of the aqueous conditions in this latter transformation and the environmental benefits of the ascorbic acid reduction prompted us to attempt to assimilate the two processes to furnish an improved protocol. We also wished to try and telescope the previous acid mediated hydrolysis of protected aniline **14** to its corresponding amino compound **20** into this new assembly. We therefore devised the set-up as depicted in Scheme 13.



Scheme 12. Flow diazotisation and Sn mediated reduction staring from aniline **20**.



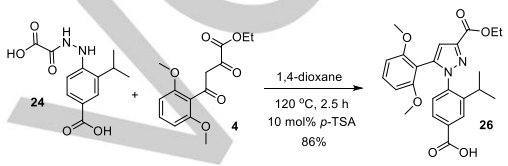
Scheme 13. Telescoped synthesis of hydrazine adduct **24**.

Taking the crude solution of acid **20** (still with **18** and **19** present) as the starting point we carried out the hydrolysis under the previously determine acidic conditions (Scheme 13). The processed flow stream then passed through a cooling zone before next being mixed with an aqueous solution of $NaNO_2$ and entering a short residence time flow coil (FEP 5 mL) maintained at 0 °C. This flow stream containing the intermediate diazonium was immediately combined with the reducing ascorbic acid (**23**) solution and the resulting hydrazine ester adduct which formed *in situ* was digested as it passed through a heated flow coil furnishing the oxamic acid **24**. To facilitate a partial workup a quench solution of NaOH was first injected into the flow to generate a basic solution followed by an input of EtOAc to form a biphasic flow. The organic and product containing aqueous stream were at this stage phase separated using the previously described in-line extractor (Figure 4). This resulted in isolation of a basic solution of the oxamic acid product **24** that could be subsequently isolated by careful acidification and extraction (of particular note was that the more aqueous soluble acid **18** was not found as a contaminant in the product).

As a proof of concept we were also able to demonstrate that this final acidification and extraction could also be achieved as a flow sequence again utilizing the in-line separator. However, in practice it was found more efficient to simply batch the product solution and acidify this in bulk prior to extraction.

Pleasingly, we achieve a reliable isolated yield of 80% both for short reactor runs (1-3 h) and over prolonged usage (full day runs of 8-10 h). This enabled a reasonable productivity of 34 mmol/h delivering over 8.9 g/h for the integrated 4 step transformation.

Finally in the context of this project we exemplified that the target hydrazine adduct **24** could be successfully utilised in the desired reaction with fragment **4** under thermal conditions to synthesise pyrazole **26** (Scheme 14). This was achieved by heating a solution of compounds **4** and **24** in 1,4-dioxane in the presence of 10 mol% *p*-TSA at 120 °C for 2.5 h. The reaction gave an 86%isolated yield of **26** after work-up and purification by column chromatography.



Scheme 14. Preparation of pyrazole **26** via batch microwave synthesis.

Conclusions

In this work we have developed a set of multi-step flow sequences starting from the readily available starting aniline **10** to an advanced hydrazine derivative **24**. The route generated has a number of staging points were natural breaks in the processing allow the batching of intermediates in each case as bench stable solids (Figure 5). As part of our flow processing strategy we have made use of some established flow transformations (e.g. diazotization and reduction) as well as developing new chemistries (e.g. selective ozonolysis of an alkyene). This has generated several improvements with regards to solvent usage (synthesis of **8** and telescoping **16**→**14**), energy utilization (synthesis of **8**) and the number of required reaction steps. Ultimately we have thus been able to create a set of linked flow operations which deliver high quality material with good productivity (Figure 5). Employing this chemistry we have successfully generated 4.27 kg of the final hydrazine derivative **24** for use in the synthesis of SR 142948A (**3**) and other important derivatives.

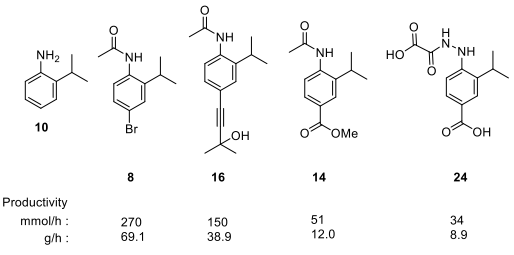


Figure 5. Key intermediates and outputs for the flow synthesis towards compound **24**.

Experimental Section

Experimental Details.

Acknowledgements

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Keywords: Flow Chemistry • Neurotensin • Ozonolysis • Sonogashira • multi-step synthesis

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