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Biomedical

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Small molecules that disrupt PCSK9•LDLR

Heart disease is the leading cause of death amongst Americans, and high cholesterol levels, particularly LDL, are pivotal in this condition. Statins, inhibitors of HMG-CoA reductase, have been the key pharmaceuticals used to lower LDL cholesterol levels. However, statin intolerance is common, and statin monotherapy is inadequate to meet targeted LDL levels for approximately 40 % of patients (*Pharmacology and Therapeutics*, 2016, j.pharmthera.2016.04.011).

Fortunately, an alternative to HMG-CoA reductase has emerged as a target for lowering LDL levels: the interaction between two proteins PCSK9 and LDLR. PCSK9 is a chaperone that binds the LDL receptor (LDLR) on the surface of liver cells. When PCSK9•LDLR captures an LDL particle, it is absorbed into lysosomes leading to metabolism of the LDL, and degradation of both PCSK9 and LDLR. However, when LDLR is not complexed with PCSK9, that receptor is recycled back to the surface of the liver cells where it is available to capture more LDL so lowering 'bad cholesterol' levels more effectively (Figure 1).

So far, mAbs have been the main strategy to disrupt PCSK9•LDLR; Sanofi/Regeneron have alirocumab and Amgen has evolocumab, and both are FDA approved for use in the US. Pfizer is hoping to soon gain approval for its mAB drug bococizumab (*amjcard*.2015.02.006).

There is enormous potential value in small molecules that can be used to disrupt PCSK9•LDLR, but surprisingly little has been published in this area, but two reports from Grazioso have been published in 2016.

In the first, his group took fragments of lupin proteins and investigated how they might inhibit the PCSK9•LDLR interaction (*Scientific Reports* 2016, srep29931). Lupin protein occurs naturally in some foods and has been associated with lowering of LDL. They do, in fact, find that some products of proteolytic degradation of the lupin protein do increase LDL uptake in a cellular assay.

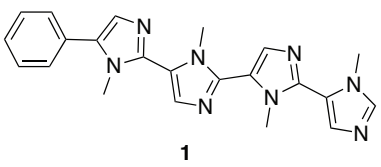


Figure 1 ***Biomedicinal relevance of inhibition of PCSK9•LDLR***

In other work, Grazioso has collaborated with a synthetic chemist, Stucchi, to develop a small molecule to perturb PCSK9•LDLR (*Org. Biomed. Chem.* 2016, c60b01642a).

Crystallography indicates the PCSK9•LDLR interface is based on interacting β-sheets, hence they devised molecule **1** as a mimic, where the N-methyl groups putatively represent the side-chain interactions.

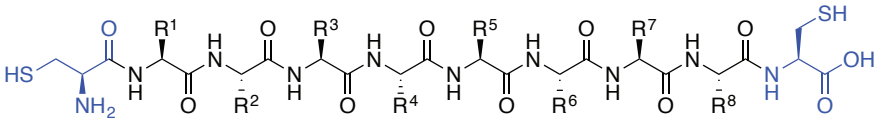
Surprisingly, micromolar concentrations of this compound impaired binding of PCSK9 to LDLR and enhanced LDL uptake in a cellular assay, even though it does not present any of the side-chains found at the PPI interface. Compound **1** is largely hydrophobic so there is a possibility that it acts via non-specific binding.

Loops, side-chains and protein-protein interactions

There has been a series of papers that collect secondary structure motifs involved in protein-protein interfaces, and attempt to identify key hot-spot residues within them using Rosetta calculations. In the latest of these Kritzer and co-workers have analysed loops at PPI interfaces (*J. Am. Chem. Soc.*, 2016, jacs.6b05656).

An upshot of this is that the authors have made available a program called *LoopFinder* that enables users to analyse loops at PPI interfaces and characterise their predicted hot-spot characteristics.

In proof-of-principle experiments,



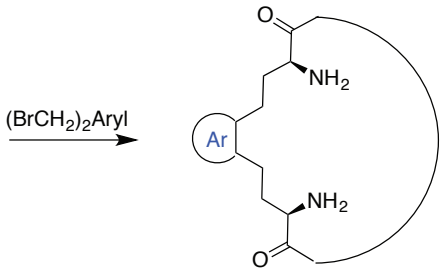
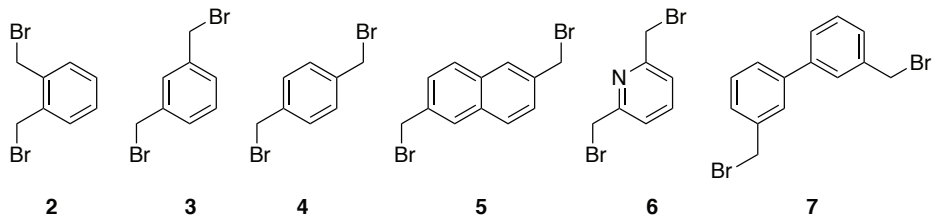


Figure 2 ***A facile cyclization strategy to make loop mimics.***



these researchers identified a loop at an interface that had multiple hot-spots (a hot-loop) and found a linear 10-mer peptide corresponding to part of this sequence had an 18 μM K_d. They then made similar 10-mer peptides wherein the C- and N-terminal residues were Cys.

When mixed with dibenzylic bromides (**2** – **7**) these unprotected peptides cyclised cleanly (~90 %) (Figure 2), hence the authors were able to identify inhibitors for the PPI that bound the protein receptor with Kd values as low as 0.33 μM.

In similar research, Arora *et al* has analysed rotations about Cα – Cβ side-chain bonds in amino acids at hotspots (*J. Am. Chem. Soc.*, 2016, jacs.6b04892). They found that rotation about this bond in hot-spot residues adopts unfavorable conformations relative to that particular side-chain in proteins in general.

They conclude: ' ...side-chain rotamers contribute significantly to the desired level of specificity in a protein-protein interaction.'

However, it is not clear if this observation is, in fact, a cause of specificity, or an effect. It could be that side-chain compromise their intrinsic preferred rotational angles about Cα – Cβ side-chains simply to accommodate the other protein at

Organic chemistry

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Dual mode organocatalyst for the axa-Sakurai cyclisation

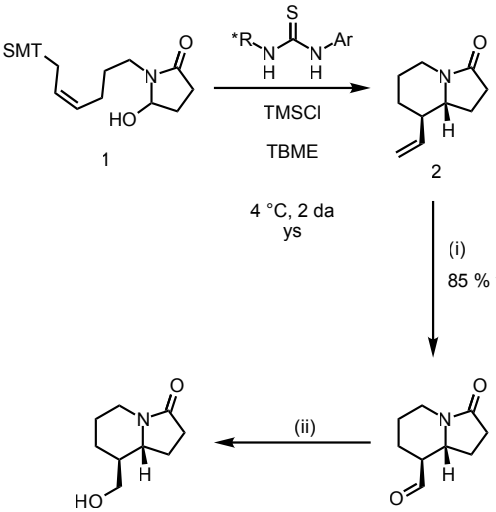
Sometimes reactions come to prominence by application in target molecule synthesis, one of the great proving grounds for the generality of synthetic methods. Jacobsen's group, with their recent synthesis of tashiromine (**3**), draw attention to the convenience of the aza-Sakurai cyclisation reaction, which produces indolizidines and quinolizidines from chlorolactams and allylsilanes (Y. Park, C. S. Schindler, E. N. Jacobsen; *J. Am. Chem. Soc.*, 2016, **138**, 14848).

Tashiromine is not a large target molecule, but it provides a nice illustration. Viewed from the stand-point of retrosynthetic analysis, lactam and ethynyl FGIs (functional group interconversions) are introduced to set up the concept of the aza-Sakurai synthetic.

In its execution to convert **1** into **2** (Scheme 1), the dual role of the thiourea catalyst holds the key. This assists the detachment of the chloride leaving group and forms a chiral ion pair with the resulting acyliminium ion. Substituents at two stereogenic centres in the chiral section of the thiourea (R*, see Figure 1) were varied in the optimisation of enantioselectivity.

An organocatalytic asymmetric aza-Piancatelli rearrangement

The aza-Piancatelli rearrangement has been modified to establish an efficient asymmetric organocatalytic version (Scheme 2) that furnishes disubstituted aminocyclopentenones



Scheme 1 ***The chiral auxiliary R* in the thiourea organocatalyst has been utilised to to access 2 in high enantiomeric excess (e.e.). The product was converted into tashiromine (3) in two steps: (i) K_2OsO_4, $NaIO_4$, THF/H_2O; and (ii) $LiAlH_4$, THF, reflux.v***

such as **6** in high enantiomeric excess (H. Li, R. Tong, J. Sun; *Angew. Chem. Int. Ed.*, 2016, 55, 15125).

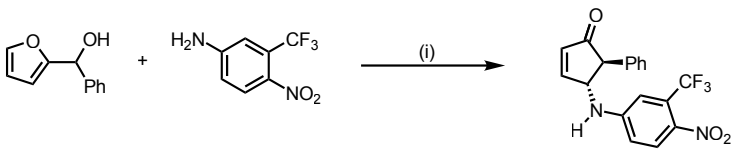
The great advantage with this method is that the starting material, a hydroxyalkyl substituted furan, is far more accessible than the product, because of the ease of acylation of furan at the 2-position. The selection of chiral auxiliaries used here are less unusual than those employed in Figure 1 for the aza-Sakurai reaction, but the importance of controlling enatioselectivity by exploiting chiral ion pairs is common in both studies.

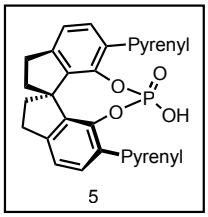
In this case, the key event that establishes the configuration of the product is the 4π conrotatary cylisation of the intermediate **6**, which also ensures the *trans* diastereoselectivity of the procedure.

A 'zip' that ends with alkyne transfer

The use of organopalladium chemistry is not dead in this era of innovation in the field of organocatalysis. Important new examples still emerge, perhaps particularly in the area of metal-promoted 'zipper'-chemistry. Palladium 'zippers' end with a σ-bound organopalladium intermediate, which in this case is intercepted by an elegant capture step that draws on the now well-established generation of copper acetylides, which is the initiation mechanism for the palladium catalysed Sonogashira coupling.

Initial π complexation of the





Scheme 2 ***Bulky pyrenyl ligands in ligand 5 (see box) give good results in the enantioselective formation of 6; (i): 5 (10 mole%), DCE, rt.***

alkyne by copper(I) lowers the p*K*a of its C-H bond sufficiently to allow mild bases to effect the deprotonation step. Instead of transmetallation at the start of the cycle, the mechanism that is proposed for the dearomative arylalkynylation of indoles **8** to form **9** effects transmetallation between the copper acetylide and the organopalladium intermediate after the σ-bond migrations of the zipper stage of the cycle have been completed, but the principle is the same (Scheme 3) (R.-R. Liu, T.-F. Xu, Y.-G. Wang, B. Xiang, J.-R. Gao, Y.-X. Jia; *Chem. Commun.*, 2016, **52**, 13664).

After transmetallation, access to the *cis* stereoisomer allows reductive elimination to complete the organic product and close the cycle by returning the palladium to the zero oxidation state.

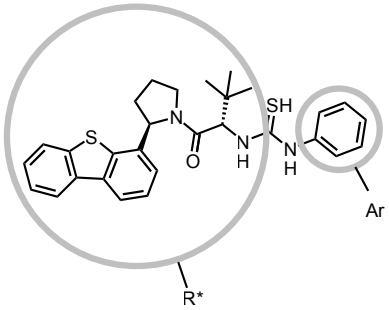


Figure 1 ***R* and Ar groups used in the reaction shown in Scheme 1. The inclusion of two stereogenic centres in R* proved to be essential for efficient reaction; when the dibenzothiophene was omitted, a racemic product was obtained in very low yield***

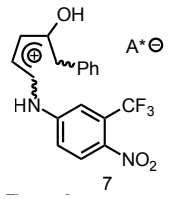
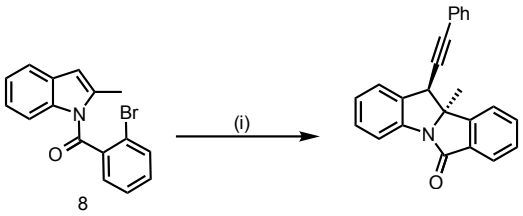


Figure 2 ***The ion pair before the stereoselective cyclisation to form 6. The acidic catalyst 5 protonates the furan oxygen ultimately producing the OH substituent in 7 and leaving the chiral phosphate dianion [A*]– to induce asymmetry by associating with the cation intermediate to form an ion pair***

Scheme 3 ***(i): Ph-C≡CH, Pd(OAc)$_2$, P^{t}Bu$_3$.HBF$_4$, CuI, ^{i}Pr$_2$NH, DMF, 100°C***



Analytical chemistry

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Speciation of silver nanoparticles

Silver nanoparticles are extensively used in consumer and medical products due to their broad spectrum antimicrobial properties. This results in them entering the environment where they undergo a range of chemical transformations, mostly resulting in the formation of silver sufide nanoparticles (AgSNPs). In order to understand the processes, speciation of the AgSNPs is vital.

Zhou, Liu, Yuan and Chen have employed pre-concentration of the AgSNPs using magnetic solid phase extraction prior to speciation by inductively coupled plasma mass spectrometry (*J. Anal. At. Spectrom.*, 2016, **31**, 2285).

Aged iron oxide magnetic particles were used for the extraction with the interference from humic acid being eliminated by the addition of Ca^{2+}. After extraction, thiourea was used to completely elute the AgSNPs prior to analysis. The limit of detection was 0.068μgL^{-1}, which was sufficient for the speciation of AgSNPs in water samples and permitted a sound understanding of the sulfidation process in the environment.

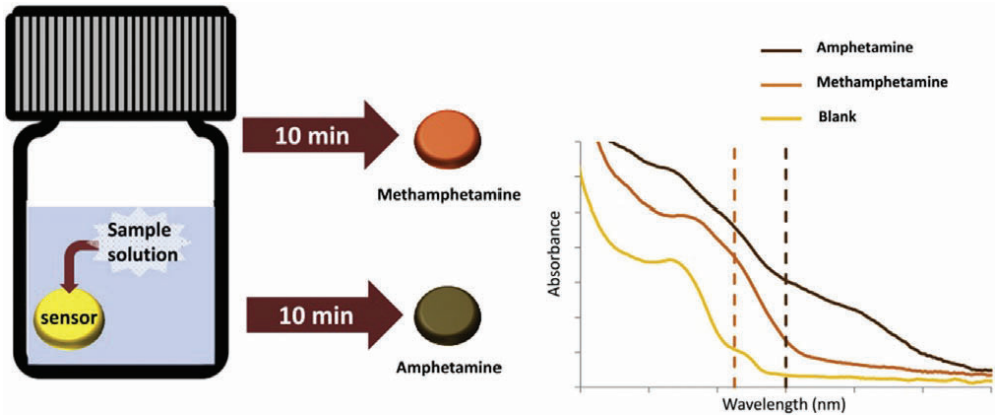

Figure 1 ***The operating principle for the colorimetric sensor***

Renewable solid electrodes

Electrochemistry is a very widely used detection method in analytical chemistry, but perhaps the greatest challenge that plagues the technique is electrode fouling. Apart from the dropping mercury electrode and one-use disposable electrodes, most if not all electrode surfaces can become fouled during repeated use. Many approaches exist to clean electrode surfaces, including electrochemical regeneration and polishing. They can have limited success and are time consuming. In microfabricated devices, practical difficulties usually preclude the most effective method of polishing.

Teixeira and co-workers have reported a method to incorporate renewable electrodes into a micro fabricated device (*Anal. Chem.*, 2016, **88**, 11199). The device was fabricated from a single block of polydimethylsiloxane (PDMS) polymer with four intersecting channels. The single fluid channel was intersected by three perpendicular electrode channels such that the fluid was able to flow around the electrodes.

The electrodes were fabricated from modified stainless steel microwires, such that they performed as working, reference and auxiliary electrodes. After each measurement, the wires were simply pulled through the channels, thereby providing a new surface for each measurement. The elastomeric nature of the PDMS device allowed for perfect seals to be maintained at flow rates up to 40mLmin^{-1}.

This approach offers a simple but reliable way to incorporate electrochemical detection into microfluidic devices without electrode fouling being a problem.

Location specific detection of cocaine on banknotes

Although cocaine can be found on many banknotes in circulation, the level is usually higher on notes that have been in close proximity to the drug and often associated with those dealing or selling drugs. Usually, the presence of cocaine on bank notes is determined by ion mobility spectrometry (IMS). Although very effective for cocaine detection, IMS lacks spatial resolution.

A method has been developed, based on in-gel bioanalytical immunodetection, which can provide spatially resolved detection of cocaine on banknotes (S. van der Heide, A. Cunningham, S. Hardwick, D. A. Russell, *Analyst*, 2016, **141**, 6116).

Banknotes were first treated with polyacrylamide to fix the cocaine in place within the gel matrix before immunostaining was performed using an anti-cocaine primary antibody which was labelled with horseradish peroxidase. The detection and location of the cocaine on the surface was achieved using chemiluminescence.

This approach allowed both the quantity and location of cocaine on bank notes to be detected and showed that the exact location of cocaine on banknotes varied between different notes.

Colorimetric detection of drugs

The problem of illegal drugs on the street remains a problem for law enforcement agencies. Many of the drugs in use are based on amphetamines and much of the analysis of these substances is laboratory based. However, the use of rapid and portable analytical tools would prove invaluable to police and customs. Although a number of such analytical processes are available, they either require expensive equipment or use potentially harmful reagents.

A simple colorimetric sensor has been developed for such applications, which can provide quantification within 10 minutes (A. Argente-Garcia, N. Jornet-Matinez, R. Herraez-Hernandez, P. Campins-Falco, *Anal. Chim. Acta*, 2016, **943**, 123).

A solid sensor element was created by embedding 1,2-naphthoquinone-4-sulfonate into a polydimethylsiloxane/tetraethylorthosilicate/silicon dioxide nanoparticles composite. Samples of drugs were placed into glass vials to which 1mL of hydrogen carbonate buffer was added, followed by the sensor. This can be seen in Figure 1. After 10 minutes, the sensor was removed and examined by diffuse reflectance spectroscopy or colour intensity measurement. The limits of detection were in the range 2 - 5mgmL^{-1} with standard deviations of less than 10%.

This method provides an excellent method for screening substances *in-situ* via a rapid, reliable and portable analytical tool.

On-line speciation sensor

Metal ions can exist in a range of different speciation states and in biological and environmental situations, this can have a big impact on how they interact with these systems. Speciation studies are complex but can be achieved via a range of measurement methodologies.

Pathirathna and co-workers have reported preliminary speciation studies, which look very promising for Cu^{2+} species (*Analyst*, 2016, **141**, 6432). They employed carbon fibre electrodes with fast scan adsorption controlled voltammetry (FSACV) to detect the Cu^{2+} species in different matrices. The electrodes were fabricated by vacuum aspirating carbon fibres of 5 µm diameter into a glass capillary of 0.4mm internal diameter before being pulled to create the usable electrode. It was possible to model the correlation between the FSACV response and the free copper concentrations for a range of copper complexes.

The results showed very great potential for the use of fast scan voltammetric techniques for speciation studies, but the most important aspect was that it is applicable to a wide range of metals in a diverse matrices.

Micro-fluidic extraction device

Conventional liquid-liquid extraction approaches remain very popular and versatile for sample preparation prior to analysis. However, due to their high solvent and sample consumption, efforts to miniaturise it continue.

Ramos-Payan, Maspoch and Llobera have reported a miniaturised

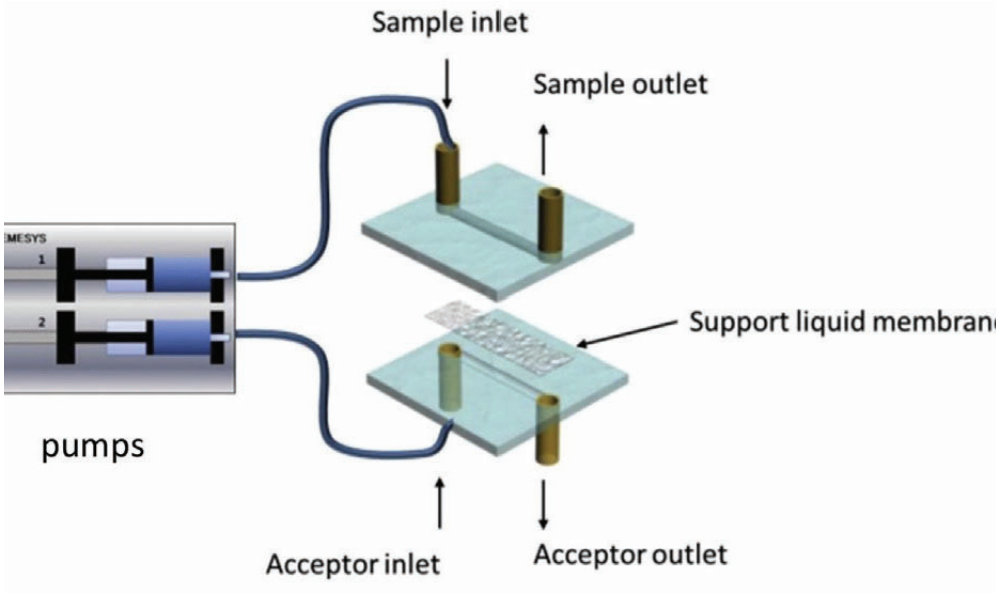

Figure 2 ***The microfluidic liquid phase micro-extraction device***

device for the extraction of non-steroidal anti-inflammatory drugs from biological and environmental samples prior to high performance liquid chromatography (HPLC) analysis (*Anal. Chim. Acta*, 2016, **946**, 56).

The device was fabricated from two sheets of poly-(methyl methacrylate) with one channel 13mm long, 2mm wide and 80µm deep in each sheet. The two channels were separated by a polypropylene liquid support membrane. The device can be seen in Figure 2. The sample solution (pH 12 flowed through one channel at 1µLmin^{-1} while the acceptor solution (pH 1.5) flowed at the same rate through the other. Extraction efficiencies of up to 87% were realised and the extracted analytes could be quickly transferred to the HPLC for analysis.

This method offers a dramatic decrease in sample and reagent consumption, typically 7µL of each; however, if the flow of acceptor solution is stopped while the sample continues to flow, much higher enrichment factors can be achieved.

Carbon fibre nanotubes in a flow-through electrochemical sensor

Analytical electrochemistry is usually a process, which occurs at the surface of an electrode with the rate at which the analyte reaches the measurement surface controlled by mass transfer processes. While in most cases this is perfectly adequate, for trace analysis applications some form of electrochemical accumulation prior to analysis is necessary.

An approach has been developed by Buffa and co-workers which uses a flow through electrode fabricated from carbon nanotubes (A. Buffa, Y. Erel, D. Mandler, *Anal. Chem.*, 2016, **88**, 11007).

Due to the greater electrode surface area, they were able to obtain far greater sensitivity and thus detect 64ppt of copper with a deposition time of 5 minutes and with a working analytical range of 10^{-9} to 10^{-5}M copper.

The work showed how different geometries of the nanotubes influenced the characteristics of the electrode and had a significant influence of the electrochemical and fluid flow properties of the electrode. Although in this case the electrode was used for trace level electroanalysis, it also has significant potential for electrochemical treatment of waste waters for the removal of trace metallic and organic pollutants.

The winning team from the 3rd National Retrosynthesis Competition, 2016

4th National Retrosynthesis Competition 2017

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