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Click Chemistry and Medicinal Chemistry: A Case of "Cyclo-*Addiction*"

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Dedicated to K. B. Sharpless

Introduction

It is fair to say that in the current climate, the concept of click chemistry (CC) perhaps requires no introduction. Most organic chemists are familiar with the term, but for many, the original meaning and philosophy have been misplaced. When the topic of click chemistry is discussed, some immediately think of the Cu^{I}-catalysed Huisgen cycloaddition as a synthetic ideal, others might consider polymer synthesis, whilst others may think of enzyme-catalysed templated reactions. Therefore, it seems pertinent to begin by reiterating the original definitions as originally laid down by the orchestrators of the concept, namely Sharpless, Finn, and Kolb.[1] It is worth remembering that the impetus behind defining the philosophy was the bleak reality that the estimated number of 'reasonable' drug candidates—those with fewer than 30 non-hydrogen atoms; with mass <500 Da composed of only H, C, N, O, P, S, F, Cl and Br; and that are likely to be stable at ambient temperature in the presence of water and oxygen—is on the order of 10^{62} molecules. Faced with this fact, it might seem clear that synthetic propositions aimed at drug discovery should be aimed at molecules that are easy to make. The rules defining a click chemistry approach are as follows: *A reaction must be modular, wide in scope, give very high yields, generate only inoffensive byproducts that are easily separated, and be stereospecific. The process must include simple reaction conditions, readily available starting materials and reagents, the use of no solvent, or a solvent that is benign or easily removed, and simple product isolation.*[1]

How much chemistry does a chemist need?

In the global chemistry community, it can be said that CC has been received with bipolar degrees of acceptance. In one camp are those who feel that the definition of click chemistry is unnecessary, that it is merely a restatement of the commonsense concerns of every synthetic chemist, who will always seek to employ the most efficient reaction for the task. In the other camp are those who, through their eager use of the principles, seemingly adore the concept. Its detractors, however, cannot pass the whole thing off as a fleeting trend in fashion, as a wealth of publications continue to be added to at an ever-increasing rate.[1]

The question heading this section was originally posed by Barry Sharpless himself at a recent conference in Berlin.[2] The answer, perhaps, is not much. Whilst CC was originally pitched as a concept to assist medicinal chemists in overcoming combinational chemistry issues, many of the publications exemplifying CC are those from materials science. The eagerness of this community to adopt CC strategies for the synthesis of polymers, dendrimers, etc., seems to reflect the attitude of '*why bother making things overcomplicated?*' Essentially a restatement of CC, the attitude is exemplified by numerous publications wherein the high-yielding reliability of the Cu^{I}-catalysed Huisgen reaction is used largely to overcome problems of low reactivity on polymer or dendrimeric scaffolds. Alongside are the medicinal chemists who choose to adopt the CC strategy, of which a brief account lies herein. Some are continuing in combinational chemistry applications, whilst others are using CC in the realisation of novel ideas which, before reliable and thermodynamically driven click reactions became available, were ill-advised. This review is intended to highlight both aspects of the union of CC and medicinal chemistry. However, this is by no means a comprehensive account of CC, and we direct the reader to related and complementary reviews in the field of CC.[3]

A final point before uncovering the medicinal applications is to address the issue of CC versus the Cu^{I}-catalysed Huisgen reaction. It is important to remember that CC was originated before the evolution of the Cu^{I} catalyst modification of the Huisgen cycloaddition, and that there are other examples of reactions that meet the CC criteria, including mainly olefin-based reactions. However, the Cu^{I} Huisgen reaction is currently the 'cream of the crop', and this is correspondingly reflected in the literature to the extent that this reaction is sometimes interpreted as CC in its entirety.

[1] *A SciFinder Scholar search for the topic 'click chemistry' indicates a yearly increase in the number of publications:* ***2002****, 6;* ***2003****, 9,* ***2004****, 43;* ***2005****, 86;* ***2006****, 213;* ***2007*** *(Nov), 274.*

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The combinatorial chemist and CC

One of the original aims of CC was to provide an alternative to solid-phase synthesis, the popularity of which was accounted by Sharpless et al. as being derived from the allowance of "*reactions that fall short of 'click' status to be employed as click reactions*".[1] High yields and simple purification are available by using high excesses of reagents and washings as opposed to conventional chromatography. The CC alternative was intended to allow large-scale solution-phase library synthesis using reliable chemical processes. Numerous groups were drawn to this alternative, and some examples of successful library synthesis are given herein. Kolb and Sharpless[4] highlight the work by researchers at Lexicon Pharmaceuticals who effected a solution-phase library synthesis using numerous CC reactions to afford 200000 individual compounds of acceptable purity on the 25–50-mg scale. Synthesis was initiated with noncommercial building blocks synthesised on a large scale. These starting materials included epoxides and aziridines ready for click nucleophilic ring opening to give 1,2-difuntionalised compounds. Imidoesters gave five-membered aromatic heterocycles from base-catalysed 1,3-dipolar cycloaddition with β-ketoesters, whilst 3-aminoazetidines gave nonaromatic heterocyclic libraries. Maintaining a CC philosophy, only one or two synthetic steps were involved in the synthesis of the library molecules, a synthesis which was carried out using automated liquid handling stations. One targeted library led to the discovery of potent peroxisome proliferator-activated receptor γ (PPAR-γ) agonists.

More recently Xie and Seto[5] synthesised a library of protein tyrosine phosphatase (PTP) inhibitors. Their approach started with the short synthesis of the α-ketoester azide compound **2** shown in Scheme 1.

The methyl ester intermediate was a necessary installation and was designed to be removed after CC library synthesis. Thus a rare instance of failure of the Cu^{I}-catalysed reaction was uncovered. It was hypothesised that compound **1** failed to react under various Cu^{I}-mediated conditions due to complexation of the metal by the bidentate α-keto acid anion. Similar complexes involving different metal ions have been reported. After solving this problem, the library was synthesised by Cu^{I}-catalysed Huisgen reaction with 56 small alkyne fragments, 54 of which were obtained commercially and the other two were synthesised in short one-step and two-step procedures. The methyl esters were then hydrolysed, and both acid and ester were screened. One hit from this screen was identified. This was re-synthesised, modified with an azide functionality, and then 'clicked' onto the same 56 alkynes to reveal a second-generation library, the result of which led to the discovery of a nanomolar inhibitor of two important PTPs. This two-stage technique led to compound **3**, which was shown to be nine-fold more potent than the original fragment **1** after the first round of 'clicking', and after the second round, to compound **4**, which is 400-fold more potent than **1** (Figure 1).

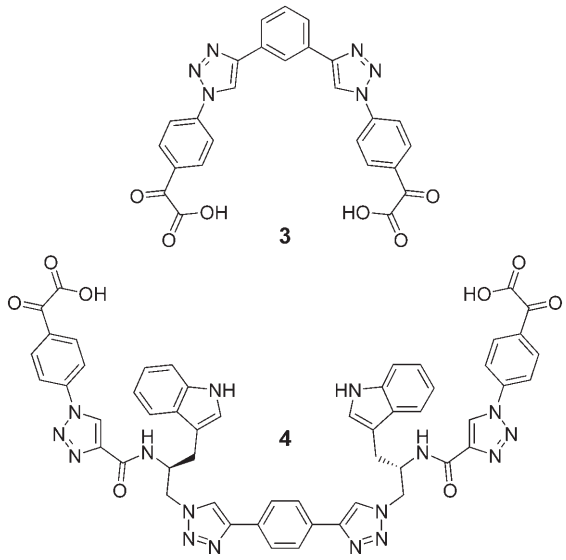
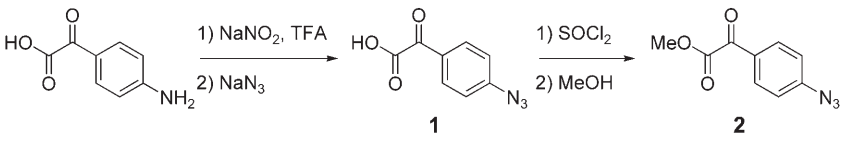


Figure 1. Two library members: **3**, a first-generation protein tyrosine phosphatase (PTP) inhibitor and **4**, a second-generation inhibitor.

In many CC library syntheses, it is fair to say that the rate-limiting steps are those preceding the final click reactions, that is, building-block synthesis. Since the widespread use of the Cu^{I}-catalysed Huisgen reaction, there have been notable advances in methodologies for the synthesis of azides. Often perceived as problematic, recent synthetic developments in azide synthesis have often been coupled with CC to give one-pot processes that circumvent the isolation of the azide building block intermediate. Examples include the use of TfN_3 as a diazo transfer reagent by Wittmann and co-workers,[6] the use of microwave irradiation to effect the synthesis of 1,2,3-triazoles via a three-component reaction reported by Van der Eycken and co-workers,[7] and also our own work using aprotic diazotisation and $TMSN_3$ to generate aromatic azides and the resultant 'click' cycloaddition products from the corresponding aniline derivatives.[8] These in situ protocols may offer some relief to the so-called 'azido-phobia', experienced by some!

Other CC libraries reported include those in which an existing lead compound or drug is decorated with either alkyne or azide functionality to create a starting point for library synthesis. Jiang and co-workers identified zanamivir (**5**) as an ideal candidate for this type of treatment.[9] Not only is the pursuit of zanamivir derivatives a valid means to identify new target compounds for the treatment of avian influenza virus (AIV), the synthetic route to zanamivir involves incorporation of azide



Scheme 1. Synthesis of the α-ketoester azide fragment **2**.

functionality as a protecting group, or as Jiang et al. realised, as a handle onto which commercial alkyne fragments could be 'clicked' to easily generate a small library of analogues. Of the 16 novel compounds synthesised, some were moderate inhibitors of AIV (H5N1), and one, compound **6** was almost as active as zanamivir (Figure 2).

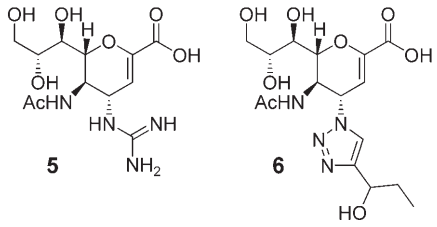


Figure 2. Zanamivir (**5**) and a synthetic compound **6**, synthesised using CC.

Although typically generating much smaller libraries, natural product analogues are readily accessible using CC. Natural product frameworks can typically contain numerous potentially reactive functionalities in their final constitution, thus presenting problems in targeting a particular handle onto which appendages are added. CC, and particularly the bio-orthogonal Huisgen reaction has presented a useful solution. Natural products exposed to the CC approach include vitamin D (**7**), analogues of which (such as **10**) may prove useful in the treatment of cancers and skin disorders;[10,11] kabiramide C (**8**), the analogue of which (compound **11**) displays strong interaction with G-actin and was shown to be as cytotoxic as the precursor itself in human cervical carcinoma cells;[12] and vancomycin (**9**), analogues of which generated through chemoenzymatic strategies coupled with CC show that rapid diversification is a feasible way to overcome the emergence of vancomycin-resistant strains of bacteria (Figure 3 and Figure 4).[13]

The aforementioned examples demonstrate the application of CC to decorate complex natural product frameworks with unnatural function, thus acting as an extremely powerful ligation tool. Some of the 'click' analogues generated from these products are shown in Figure 4. Sun, Wang, and co-workers[14] synthesised a handful of bisdaunorubicins with linkers of varying length by using the Cu^{I}-catalysed Huisgen reaction between complementary alkyne- or azide-functionalised daunorubicins, or bisacetylene- or bisazide-functionalised linkers (phenyl and PEG) to generate compounds such as **13** (Figure 5), which display differential anticancer properties towards leukaemia K562 cells, depending on the linker length and flexibility between the two intercalators.

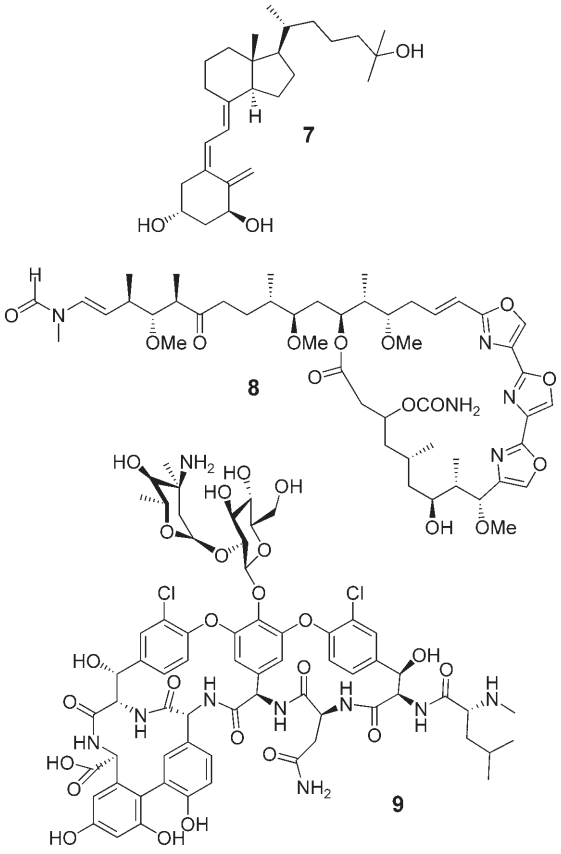


Figure 3. The natural products vitamin D (**7**), kabiramide C (**8**), and vancomycin (**9**).

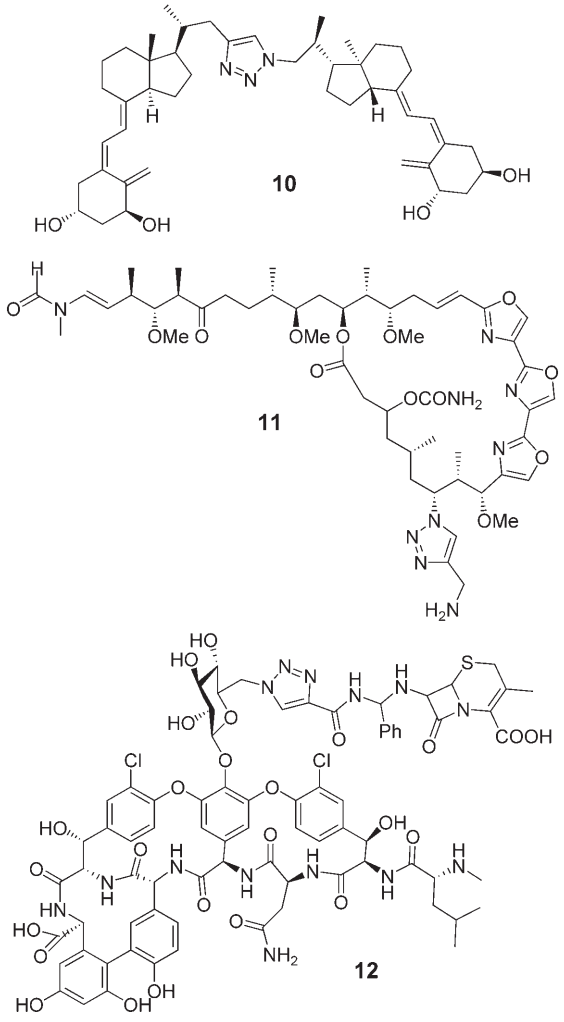


Figure 4. Examples of natural product CC 'hybrids' produced from vitamin D (compound **10**), kabiramide C (compound **11**), and vancomycin (compound **12**).

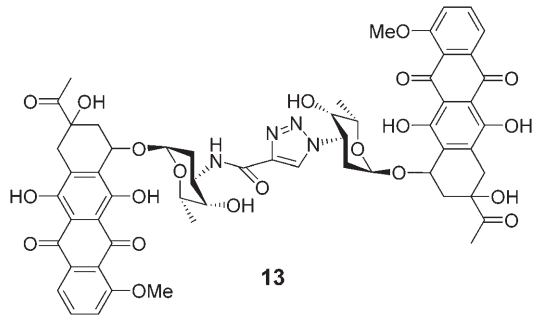
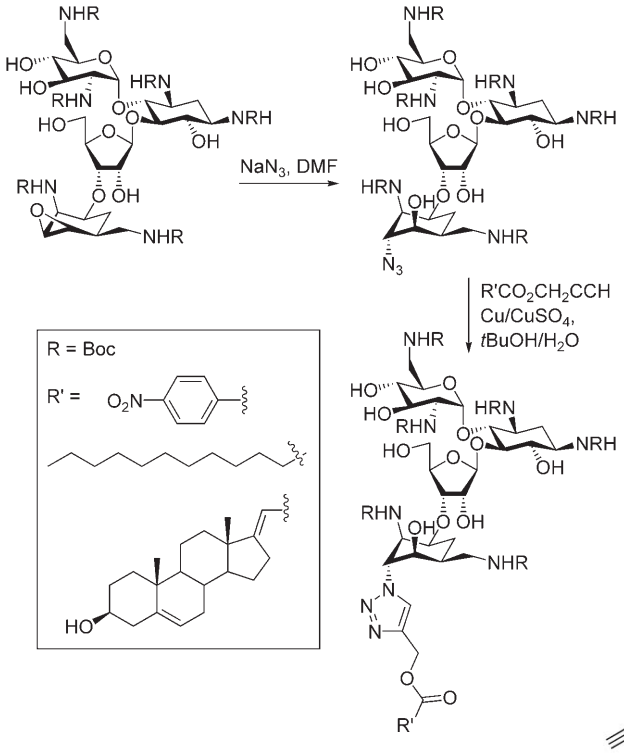


Figure 5. A novel bisdaunorubicin molecule containing a triazole CC linker.

More recently, Jenkins, Houston, and co-workers[15] employed CC in the modification of a neomycin B core (Scheme 2). Mitsunobu and epoxide chemistries furnished



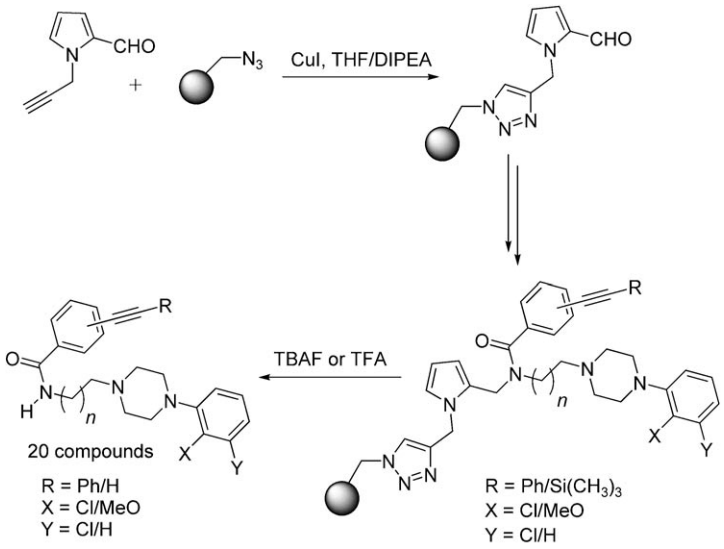
Scheme 2. An example synthetic sequence of CC modification of a neomycin core in work carried out by Houston and co-workers.[15]

azido-functionalised neomycin, which was subsequently elaborated using CC and three different alkynes designed to add lipophilic properties to the neomycin analogues. Semisynthetic analogues of this class of aminoglycosides are medicinally attractive due to the broad spectrum of activities towards a variety of RNA sequences, including those in bacterial ribosomes (thus resulting in bacterial cell death), as well as other biologically relevant RNAs such as group I introns, hammerhead ribozymes, and the hepatitis delta virus (HDV) ribozyme. Neomycin B was recently shown to be the most potent inhibitor of the proteolytic activity of anthrax lethal factor (LF).[15] Previous examples of neomycin B modification required multiple protection and deprotection steps at the numerous amino and hydroxy groups present.

Others, not giving up on solid-phase combinatorial synthesis altogether, saw the CC approach to library synthesis in a slightly different light. The reliable chemistry has been used in the development of triazole-containing linkers[16] and as a traditional solid-phase reaction. Recent examples include the work of Gmeiner and co-workers,[17] who employed pyrrole-2-carbaldehyde functionalised at the *N*-indole atom with a propargylic handle, which 'clicked' onto an azide-functionalised resin with complete loading. The aldehyde handle was then used to synthesise a focused library of compounds, some of which displayed high binding affinities to dopamine D3 and D4 receptors (Scheme 3). The same research group had previously reported the use of the Huisgen reaction in the design of both the linker and the functionalisation of solid-phase-bound alkynes to generate a library of *N*-benzyltriazole carboxamides, some of which had nanomolar affinities towards G-protein-coupled receptors.[18]

Convergent click libraries

The libraries described above were those generated from one starting unit, followed by subsequent linear divergent syntheses. We now discuss some examples of CC in the synthesis of convergent chemical libraries.[19] In convergent library synthesis, complex building blocks are coupled together at a late stage to afford higher-order products with increased diversity. A reaction that embodies the CC principles will necessarily be chemoselective, as Sharpless originally observed, "*although click reaction components are necessarily highly reactive, their chemoselectivity profiles are quite narrowly defined, that is 'orthogonal', to an unusually broad range of reagents, solvents, and*



Scheme 3. Solid-phase CC synthesis of a focused library of dopaminergic phenylacetylenes.

other functional groups".[1] Fortunately, azide and alkyne functionalities are protected by a high kinetic barrier to reaction with other functionalities, and their high thermodynamic potential may be released only when suitable catalysis (or physical restraint) allows reaction. Thus, the Cu^{I} Huisgen reaction can almost be universally relied upon to provide successful late-stage coupling regardless of the chemical functionality in the coupling fragments, a necessity in the convergent approach.

Yao and co-workers synthesised a library of bidentate inhibitors of protein tyrosine phosphatases.[20] Previous studies had indicated that peripheral binding to a second site could increase the potency and selectivity of compounds. Using this knowledge the group selected a known core binding moiety, synthesised a handful of alkyne-substituted variants of this core group (Figure 6), and then coupled them with a number of aryl azides to generate the final compounds. The library screen identified a specific PTP1B inhibitor with moderate inhibition similar to Abbott's original basis molecule. The same group also synthesised a library of matrix metalloprotease (MMP) inhibitors using an identical strategy, this time combining eight alkyne zinc binding succinyl hydroxamates with 12 azide fragments to give a library of 96 compounds,[21] some of which displayed good potency and moderate selectivity for MMP-7 over other metalloproteases.

In situ library screening

Because it is possible to carry out Cu^{I}-catalysed reactions on the micro scale in solution, the products can be subsequently screened directly (provided the catalysts are benign) without purification, where concentrations are derived by assuming a reaction yield of 100%. A general approach has been described by Wong and co-workers.[22] Initially, a test is carried out to determine whether the reaction conditions are benign to the inhibition assay. Following this, microplate reactions are performed, typically at high (5 mM) concentration. Upon completion (determined by LC–MS or TLC) the plate wells are diluted (micromolar range), and any well in which the contents cause $>50\%$ inhibition are diluted further (nanomolar range). A second screen is then carried out to reveal the potent inhibitors in the library. An impressive claim made by the authors is that with this approach, library generation (50–100 compounds) and in situ screening can be accomplished within a single day. These timescales are a massive improvement over conventional parallel synthesis and purification, thus greatly accelerating the process of lead identification. There are several reactions that have demonstrated applicability to this treatment, including amide bond formation, the Pictet–Spengler reaction, tetrabutylammonium fluoride assisted alkylation, and epoxide opening in water. The Huisgen reaction seemingly heads up this group as a reaction that appropriately works best in aqueous solution and gives 1,2,3-triazole products in high yield, purity, and regioselectivity. Moreover, this reaction, unlike the others, tolerates virtually all functionality without protecting groups. It is these factors that make it the most popular choice when envisaging a library synthesis to be screened in situ.

In their aforementioned convergent library synthesis, Yao and co-workers were able to screen their compounds for testing directly from the reaction mixture without the need for further purification.[20] Wong and co-workers have described two examples in which they successfully used the Cu^{I}-catalysed Huisgen reaction in library synthesis and followed this by screening the compounds in situ. In their realisation of inhibitors of human α-1,3-fucosyltransferases (Fuc-Ts), with little structural data available, they first identified the importance of the binding energy derived from the guanosine diphosphate (GDP) moiety of the GDP-fucose cofactor, and then synthesised a GDP core decorated with alkyne functionality (compound **15**, Figure 7).[23] This core was then treated with a library of 85 azide molecules in individual wells of a microtiter plate under Cu^{I} catalysis conditions. The GDP triazole compounds were screened for inhibitory effects against Fuc-T directly in the plates. The best performing compound **16** showed inhibition that was an 800-fold improvement over the original alkyne GDP fragment (62 nM versus 47 μM). As a tribute to this technique, **16** is the first nanomolar inhibitor of Fuc-Ts. The same research group also used the in situ screening technique in the discovery of HIV-1 protease inhibitors.[24]

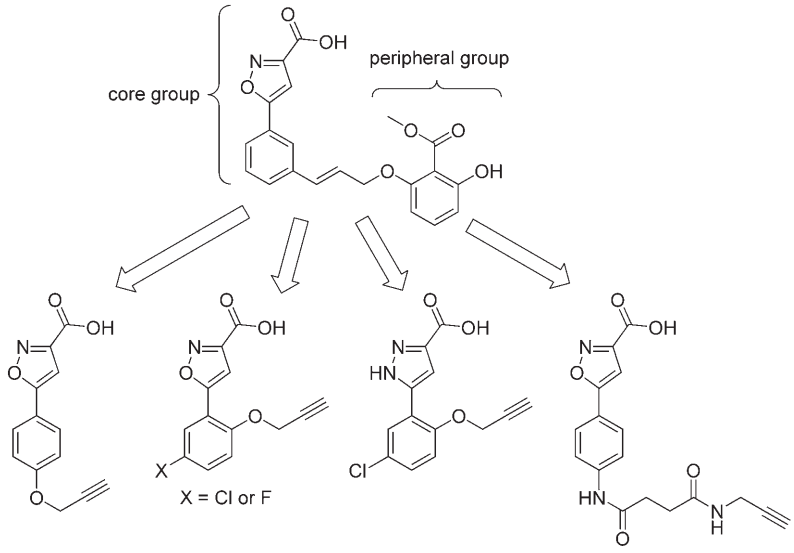


Figure 6. Design of alkyne fragments for use in the convergent library synthesis of bidentate PTP inhibitors based on Abbott's cell-permeable, bidentate PTP1B inhibitor **14**.

In situ CC

It is fair to say that click chemistry performed in situ—one par-

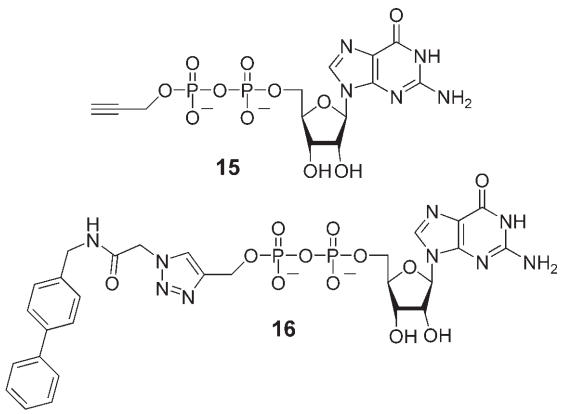


Figure 7. Alkyne fragment **15** used in in situ screening, and **16**, a potent lead discovery as a result of the screen.

ticular exponent of target-guided synthesis—is one of the jewels in the crown of CC. Although still in the stages of development, it is certainly making headlines and is one of the examples in which CC really is a beacon of state-of-the-art science. Discussion at this point follows appropriately from the combinatorial strategies previously introduced. Firstly, we discussed how CC could drop straight into the synthetic toolbox of the standard combinatorial chemist, essentially boiling down to a highly reliable reaction that can be performed simply with excellent results. Progressing from this topic, we saw the orthogonality of the azide and alkyne functionalities in the Cu^{I}-catalysed Huisgen reaction as a useful tool for the synthesis of slightly more complicated convergent chemical libraries, effective by avoiding the use of protecting groups in the synthesis of the fragments. The ability of CC to withstand more complex chemical scenarios was exemplified by the demonstration that minimal by-products, simple or no purification, and high yields (all stipulations of a CC reaction), allowed the synthesis and screening of product mixtures (typically consisting of product and catalyst) directly in microtiter plates. Herein we disclose one of the ultimate facets of the Huisgen reaction: bio-orthogonality of starting materials (and products) that is sufficient to allow the reaction to be carried out in the presence of templating enzymes and with concomitant catalysis provided by the enzyme itself, thus allowing the enzyme to be its own combinatorial chemist! Before a discussion of the details of the in situ CC approach, it is pertinent to overview the main alternatives and predecessors to in situ CC, including dynamic combinatorial chemistry (DCC) and irreversible/kinetic target-guided synthesis. Dynamic covalent chemistry is a concept originally pioneered by Lehn and others,[25] and is used in the generation of a virtual covalent library, whereby all possible bonds between a mixture of reversibly reactive fragments are formed and broken. The complex equilibrium produced in the presence of a large number of fragment molecules is the virtual covalent library. Lehn showed that the equilibrium of such a library could be perturbed by the addition of a templating enzyme, namely carbonic anhydrase, in a process that effectively usurps the synthesis of the entire library; as the enzyme template selects its preferred inhabitants from the mixture, these compounds 'selected' by the templating structure can then be assed for inhibitory effects. Following this original experiment, numerous other DCCs have been used in the synthesis of some novel inhibitors of various enzymes and secondary DNA structures, using chemistries such as imine and disulfide formation.

Irreversible/kinetic target-guided synthesis (under which the banner of in situ CC falls) effectively offers the same overall outcome, in that an enzyme selects its favourite inhibitors from a potential library. In the irreversible approach, however, the library is not synthesised by DCC; here the enzyme selects its favourite inhibitor by synthesising it itself. This approach to in vitro combinatorial chemistry has been previously attempted by different groups, employing different connecting reactions and strategies. Benkovic, Boger, and co-workers have described the tight binding of a reactive inhibitor to the enzyme active site after an enzyme-templated epoxide opening reaction with a nucleophilic site on the substrate.[26]

The use of CC for irreversible target-guided synthesis was pioneered by Sharpless et al. Despite the extremely slow rate of the Huisgen reaction at room temperature, Mock et al. had previously demonstrated that sequestration of azide and alkyne fragments inside a cucurbituril template dramatically increased the rate of cycloaddition.[27] It should also be noted that an enzyme target stabilises the transition state of a target-accelerated reaction; a product-like transition state is necessary if the accelerated products are to be successful inhibitors. Therefore, cycloaddition reactions in which the transition state does indeed reflect the structure of the product are ideal. A strategy based on the Huisgen reaction involving both azide and alkyne building blocks would elude the use of reacting species that are nucleophilic and electrophilic and consequently prone to undesired reactions with biological molecules. Encouraged by these facts, Sharpless and co-workers set about a proof-of-principle experiment involving the use of acetylcholinesterase (AChE) as their 'reaction vessel' (Figure 8).[28] This enzyme was chosen because of the availability of established inhibitors, it is known to bind both the active centre and a peripheral site, and the fact that inhibitors that span both the active centre and the peripheral site show tighter binding than the individual fragments. In a reaction time of 6 days, negligible amounts of product were formed in the absence of catalyst, but when the reaction was carried out in the presence of the enzyme, any product formed was the result of a rate-accelerating enzyme-templating effect. The result of this study of binary mixtures of all combinations of azide and alkyne, and tacrine and phenanthridinium, was the in situ discovery of a femtomolar inhibitor of AChE, making it the most potent non-covalent inhibitor of AChE known by approximately two orders of magnitude. This original work validated the use of the Huisgen reaction for the in situ assembly of molecules.

The most recent development in the in situ CC story is a very important one. Not only does it turn the attention to another, this time very relevant enzyme, but it also demonstrates that the in situ CC process can be applied to systems without the comfort of previously reported binding fragments. Previous forays have always used at least one high-affinity fragment,

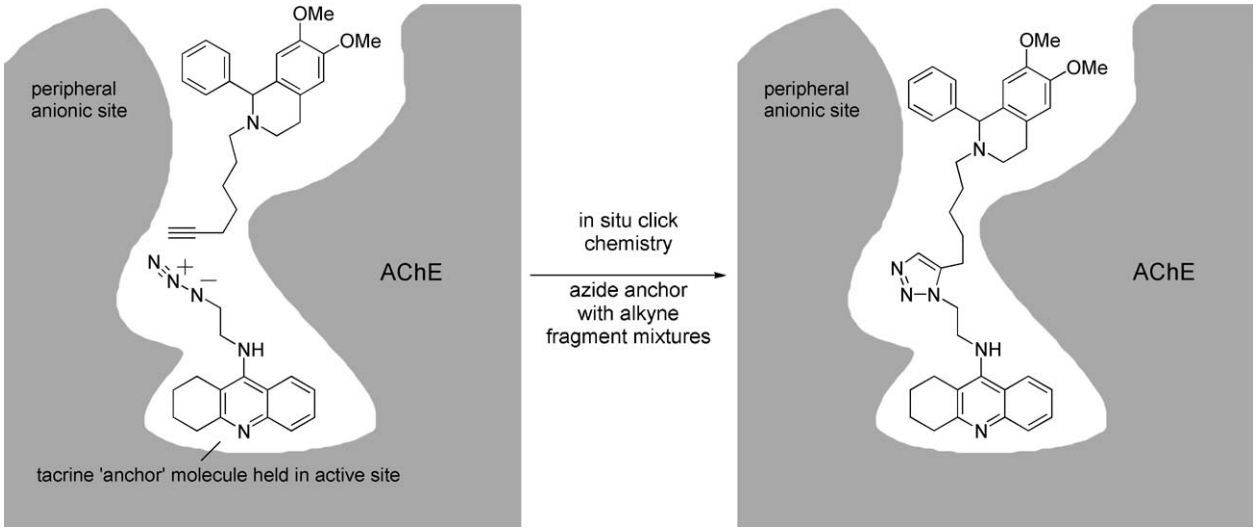


Figure 8. Schematic representation of the enzyme-templated CC reaction between a tacrine anchor azide molecule and an alkyne fragment.

which essentially anchors that fragment into the active site. Then, a large excess of the corresponding coupling fragments were used. These two factors greatly increase the kinetic and thermodynamic likelihood of successful in situ reaction. In the studies directed towards HIV-1 protease inhibitors, there were no good binding fragments available. However, Sharpless and co-workers demonstrated that an inhibitor could be assembled by using the in situ process from only weakly binding fragments, thus greatly expanding the scope of this technology to other enzymatic systems, making it a feasible tool for routine drug searches.[29] The CC approach offers a bio-orthogonal reaction with no auxiliary reagents or catalysts required, and it has been demonstrated that the triazole may also serve as more than merely a linking functionality; it may contribute to the enthalpic stabilisation of the enzyme–inhibitor complex.

A technical advance in the in situ approach has been the incorporation of the existing in situ bCAII library designed by Sharpless et al. into a microfluidic chip device, an accomplishment achieved by Kolb, Tseng and co-workers.[30] The microfluidic chip device greatly decreases the quantities of reagents required, and operates at reaction volumes of approximately 4 μL. In this particular application, this means that much smaller quantities of enzyme are required. The microfluidic chip is capable of carrying out 32 reactions in parallel. It was shown that when identical azide fragments from the original study were mixed with the acetylene anchor with an incubation time of 40 h, the microfluidic chip gave a very similar outcome to the original study. Thus the advantages of the in situ approach to lead discovery can be coupled with the use of a microfluidic chip, incorporating the advantages of low reagent consumption, precise control over reaction conditions, faster reaction kinetics, and cost efficiency to make the process potentially even more efficient.

Other applications in medicinal chemistry

The majority of this review has been concerned with displaying the work achieved based on the original intention of CC, that is to assist the medicinal chemist. However, we now focus our attention away from big libraries and high-throughput screens of combinatorial chemistry and take a brief look at the effect that CC has had on other aspects of medicinal chemistry. Not discussed herein, but well covered elsewhere, a CC approach with the Cu^{I}-catalysed Huisgen reaction has proven very useful in the conjugation of biomolecules to one another, for example in the synthesis of glycoconjugates and appending molecules to other proteins and DNA templates.[3a]

Indeed the triazole is not a new player in the arena of drug design, and as pointed out by Genazzani and co-workers, previous to the onslaught of CC there were medicinally relevant molecules containing triazole function.[31] The triazole functionality now derived from CC and the Huisgen reaction itself can fit in to the rational design of drug compounds. We have previously discussed the work of Sharpless et al., wherein the serendipitous discovery that the ligating triazole functionality inadvertently imparted their AChE inhibitor molecules with greater enthalpic stability through hydrogen bonding in the binding site, and consequently with greater inhibition, demonstrates that the triazole can serve as an effective pharmacophore. In our own work, we considered whether we could employ CC and incorporate the resulting triazole linkers as pharmacophores in G-quadruplex ligands. The G-quadruplex is a novel anticancer target, and stabilisation of this elegant DNA tertiary structure using small molecules has been shown to inhibit the action of the telomere replicating enzyme telomerase, activated in tumour cells. We successfully synthesised a small collection of molecules containing five contiguous aromatic moieties, two of which were triazoles produced from the Cu^{I}-catalysed Huisgen reaction, as their pharmacophore units.[32] The compounds were shown by fluorescence resonance energy transfer (FRET) to stabilise the quadruplex secondary

structure of guanine-rich DNA, and they were also shown to achieve good stabilisation despite high concentrations of competing duplex DNA, outperforming in this respect the gold standard of synthetic quadruplex ligands, BRACO-19 (Figure 9).

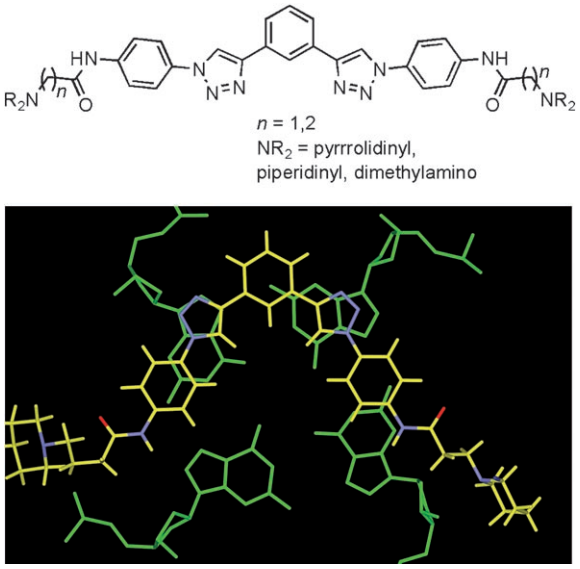


Figure 9. General structure of our 1,2,3-triazole-containing G-quadruplex-stabilising ligands, and a qualitative molecular model of compound **33** (with C, H atoms colored yellow), interacting with the G-quartet at the end of a unimolecular quadruplex structure. Only the terminal G-quartet is shown, colored green.

Others have demonstrated that the triazole can serve as a bioisostere for various functionalities including amide, *E* olefins and perhaps phosphate linkages.[33] The use of bioisosteres in the functionalisation of natural products presents the advantages of stability, biostability, and opens the door to libraries of hit compounds.

The most popular functionality for which the triazole functionality can substitute as a bioisostere is the amide bond. The triazole mimics the topology and electronic features of the amide bond, and can likewise participate in hydrogen bonding and dipole–dipole interactions. Recently, Kim et al. completed the bioisosteric replacement of the amide linkage found in ceramide **17** with the non-hydrolysable 1,2,3-triazole to produce a number of derivatives (such as **18** and **19**), the cytotoxic activities of which were greater than those of C2-ceramide[34] (Figure 10). Furthermore, for the previously mentioned HIV-1 inhibitors produced in the library synthesis of Wong et al.,[24] it was later shown (by solving the crystal structure of the inhibitors in complex with HIV-1 protease) that the triazole formed hydrogen bonds in a conformation identical with that of the amide bond in amprenavir (the original drug before modification; Figure 11).[35]

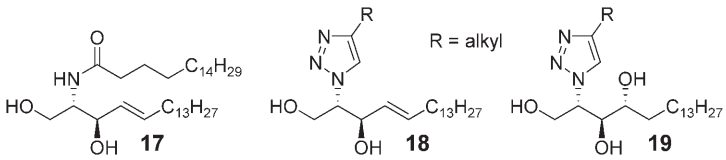


Figure 10. Ceramide **17** and some CC analogues **18** and **19**.

Previous examples given in this review have largely concerned the Huisgen reaction and its use in intermolecular reactions as a tool for the ligation of two fragment molecules. Now we describe the growing use of CC in ring-closing reactions of macrocycle synthesis. Burke, Jr. and co-workers used the Cu^{I}-catalysed reaction to achieve macrocyclisation of an azide and terminal acetylene functionalised molecule to form Grb2 SH2 domain binding macrocycles.[36] The product distribution between monomer and dimer was strongly dependent on substrate concentration. At higher concentrations the authors were able to obtain the dimer as the major product, and at lower concentrations, cyclisation of monomeric units occurred. The monomeric (*S*)-Pmp-containing product exhibited submicromolar binding affinity to the Grb2 SH2 domain (Pmp = 4-(phosphonomethyl)phenylaniline, a phosphatase-stable phosphotyrosine mimetic). Wang and co-workers also used the same reaction to form conformationally constrained macrocyclic peptidomimetic inhibitors of STAT 3.[37] In comparison with linear peptidomimetics, macrocyclic varieties can be more resistant to protease degradation, and they may also exhibit higher binding affinities (Figure 12).

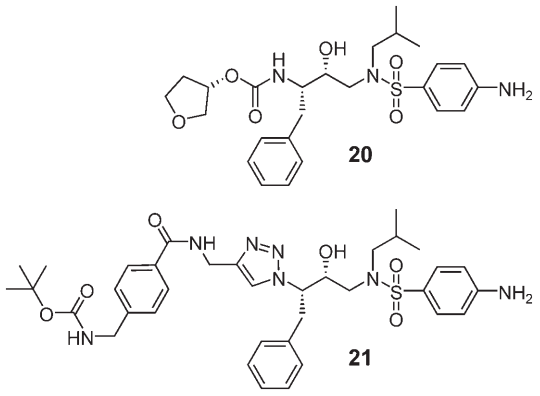


Figure 11. Amprenavir (**20**) and a CC analogue **21**.

Outlook

We have discussed numerous arenas in which CC is emerging as an extremely useful tool for the medicinal chemist. In the chemistry discussed in the final section of this review, natural product funtionalisation and bioisosteric replacement of functionality in natural products and drug targets unlocks a wealth of potential applications in drug discovery.

This review examines how the application of CC has evolved since its original conception, and as new applications continue to appear, we can assume that this trend towards increasing diversity of application will continue. With the discovery of new reactions that meet 'click' status, the horizons will be expanded even further.

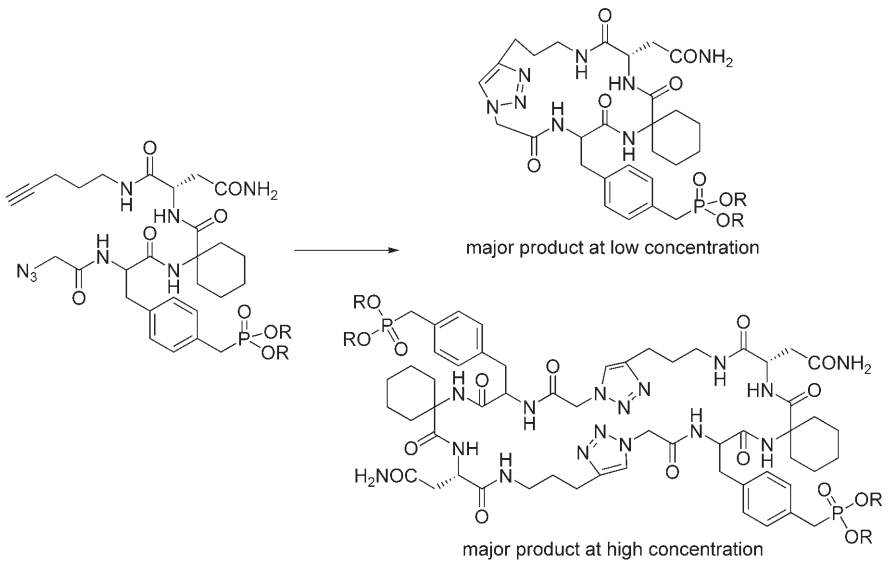


Figure 12. Grb2 SH2 domain binding macrocycles.

Keywords: click chemistry · cycloaddition · drug discovery · medicinal chemistry

[1] H. C. Kolb, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2001**, *113*, 2056–2075; *Angew. Chem. Int. Ed.* **2001**, *40*, 2004–2021.

[2] The question was discussed in K. B. Sharpless's talk at the Eighth Tetrahedron Symposium 50th Anniversary Meeting in Berlin, June 27 – 29, **2007**.

[3] a) J. E. Moses, A. D. Moorhouse, *Chem. Soc. Rev.* **2007**, *36*, 1249–1262; b) P. Ball, *Chem. World* **2007**, *4*, 46–48; c) J. F. Lutz, *Angew. Chem.* **2007**, *119*, 1036–1043; *Angew. Chem. Int. Ed.* **2007**, *46*, 1018–1025; d) A. Dondoni, *Chem. Asian J.* **2007**, *2*, 700–708; e) Y. L. Angell, K. Burgess, *Chem. Soc. Rev.* **2007**, *36*, 1674–1689; f) M. V. Gil, M. J. Arevalo, O. Lopez, *Synthesis* **2007**, 1589–1620; g) P. Wu, V. V. Fokin, *Aldrichimica Acta* **2007**, *40*, 7–17; h) W. H. Binder, C. Kluger, *Curr. Org. Chem.* **2006**, *10*, 1791–1815; i) V. D. Bock, H. Hiemstra, J. H. van Maarseveen, *Eur. J. Org. Chem.* **2006**, 51–68.

[4] H. C. Kolb, K. B. Sharpless, *Drug Discovery Today* **2003**, *8*, 1128–1137.

[5] J. Xie, C. T. Seto, *Bioorg. Med. Chem.* **2007**, *15*, 458–473.

[6] a) P. B. Alper, S.-C. Hung, C.-H. Wong, *Tetrahedron Lett.* **1996**, *37*, 6029–6032; b) H. S. G. Beckmann, V. Wittmann, *Org. Lett.* **2007**, *9*, 1–4.

[7] P. Appukkuttan, W. Dehaen, V. V. Fokin, E. Van der Eycken, *Org. Lett.* **2004**, *6*, 4223–4225.

[8] K. Barral, A. D. Moorhouse, J. E. Moses, *Org. Lett.* **2007**, *9*, 1809–1811.

[9] J. Li, M. Zheng, W. Tang, P-L. He, W. Zhu, T. Li, J-P. Zuo, H. Liu, H. Jiang, *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5009–5013.

[10] B.-C. Suh, H. Jeon, G. H. Posner, S. M. Silverman, *Tetrahedron Lett.* **2004**, *45*, 4623–4625.

[11] P. L. Suarez, Z. Gándara, G. Gómez, Y. Fall, *Tetrahedron Lett.* **2004**, *45*, 4619–4621.

[12] C. Petchprayoon, K. Suwanborirux, R. Miller, T. Sakata, G. Marriott, *J. Nat. Prod.* **2005**, *68*, 157—161.

[13] X. Fu, C. Albermann, C. Zhang, J. S. Thorson, *Org. Lett.* **2005**, *7*, 1513–1515.

[14] G. Zhang, L. Fang, L. Zhu, D. Sun, P. G. Wang, *Bioorg. Med. Chem.* **2006**, *14*, 426–434.

[15] S. Quader, S. E. Boyd, I. D. Jenkins, T. A. Houston, *J. Org. Chem.* **2007**, *72*, 1962–1979.

[16] S. Löber, P. Rodriguez-Loaiza, P. Gmeiner, *Org. Lett.* **2003**, *5*, 1753–1755.

[17] P. Rodriguez-Loaiza, S. Löber, H. Hübner, P. Gmeiner, *Bioorg. Med. Chem.* **2007**, *15*, 7248–7257.

[18] P. Rodriguez-Loaiza, S. Löber, H. Hübner, P. Gmeiner, *J. Comb. Chem.* **2006**, *8*, 252–261.

[19] For a review see: A. B. Beeler, S. E. Schaus, J. A. Porco, Jr., *Curr. Opin. Chem. Biol.* **2005**, *9*, 277–284.

[20] R. Srinivasan, M. Uttamchandani, S. Q. Yao, *Org. Lett.* **2006**, *8*, 713–716.

[21] J. Wang, M. Uttamchandani, J. Li, M. Hu, S. Q. Yao, *Org. Lett.* **2006**, *8*, 3821–3824.

[22] A. Brik, C.-Y. Wu, C.-H. Wong, *Org. Biomol. Chem.* **2006**, *4*, 1446–1457.

[23] L. V. Lee, M. L. Mitchell, S.-J. Huang, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *J. Am. Chem. Soc.* **2003**, *125*, 9588–9589.

[24] A. Brik, J. Muldoon, Y.-C. Lin, J. H. Elder, D. S. Goodsell, A. J. Olson, V. V. Fokin, K. B. Sharpless, C.-H. Wong, *ChemBioChem* **2003**, *4*, 1246–1248.

[25] a) I. Huc, J.-M. Lehn, *Proc. Natl. Acad. Sci. USA* **1997**, *94*, 2106–2110; b) J.-M. Lehn, *Chem. Eur. J.* **1999**, *5*, 2455–2463; c) S. Otto, R. L. E. Furlan, J. K. M. Sanders, *Drug Discovery Today* **2002**, *7*, 117–125.

[26] S. E. Greasley, T. H. Marsilje, H. Cai, S. Baker, S. J. Benkovic, D. L. Boger, I. A. Wilson, *Biochemistry* **2001**, *40*, 13538–13547.

[27] a) W. L. Mock, T. A. Irra, J. P. Wepsiec, T. L. Manimaran, *J. Org. Chem.* **1983**, *48*, 3619–3620; b) W. L. Mock, T. A. Irra, J. P. Wepsiec, M. Adhya, *J. Org. Chem.* **1989**, *54*, 5302–5308; c) W. L. Mock, *Top. Curr. Chem.* **1995**, *175*, 1–24.

[28] W. G. Lewis, L. G. Green, F. Grynszpan, Z. Radiæ, P. R. Carlier, P. Taylor, M. G. Finn, K. B. Sharpless, *Angew. Chem.* **2002**, *114*, 1095–1099; *Angew. Chem. Int. Ed.* **2002**, *41*, 1053–1057.

[29] M. Whiting, J. Muldoon, Y.-C. Lin, S. M. Silverman, W. Lindstrom, A. J. Olson, H. C. Kolb, M. G. Finn, K. B. Sharpless, J. H. Elder, V. V. Fokin, *Angew. Chem.* **2006**, *118*, 1463–1467; *Angew. Chem. Int. Ed.* **2006**, *45*, 1435–1439.

[30] J. Wang, G. Sui, V. P. Mocharla, R. J. Lin, M. E. Phelps, H. C. Kolb, H.-R. Tseng, *Angew. Chem.* **2006**, *118*, 5402–5407; *Angew. Chem. Int. Ed.* **2006**, *45*, 5276–5281.

[31] G. C. Tron, T. Pirali, R. A. Billington, P. L. Canonico, G. Sorba, A. A. Genazzani, *Med. Res. Rev.* **2007**, DOI: 10.1002/med.20107.

[32] A. D. Moorhouse, A. M. Santos, M. Gunaratnam, M. Moore, S. Neidle, J. E. Moses, *J. Am. Chem. Soc.* **2006**, *128*, 15972–15973.

[33] a) R. V. Somu, H. Boshoff, C. Qiao, E. M. Bennett, C. E. Barry III, C. C. Aldrich, *J. Med. Chem.* **2006**, *49*, 31–34; b) F. Pagliai, T. Pirali, E. Del Grosso, R. Di Brisco, G. C. Tron, G. Sorba, A. A. Genazzani, *J. Med. Chem.* **2006**, *49*, 467–470.

[34] S. Kim, M. Cho, T. Lee, S. Lee, H-Y. Min, S. K. Lee, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 4584–4587.

[35] A. Brik, J. Alexandratos, Y. -C, Lin, J. H. Elder, A. J. Olson, A. Wlodawer, D. S. Goodsell, C.-H. Wong, *ChemBioChem* **2005**, *6*, 1167–1169.

[36] W. J. Choi, Z.-D. Shi, K. M. Worthy, L. Bindu, R. G. Karki, M. C. Nicklaus, R. J. Fisher, T. B. Burke, Jr., *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5265–5269.

[37] J. Chen, Z. Nikolovska-Coleska, C.-H. Yang, C. Gomez, W. Gao, K. Krajewski, S. Jiang, P. Roller, S. Wang, *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3939–3942.

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