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ARTICLE

Diversifying Chemical Space of DNA-Encoded Libraries: Synthesis of 2-Oxa-1-azaspiro(bicyclo[3.2.0])heptanes On-DNA via Visible Light-Mediated Energy Transfer Catalysis

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Azaspiro[3.3]heptanes are valuable synthetic targets for drug discovery programs. The challenges associated with the preparation and diversification of this moiety as compared to other small, saturated rings have led to limited applications of compounds containing this spirocycle. In this regard, important advances in the field of synthetic photochemistry have exploited the biradical nature of the triplet excited state of 2-isoxazoline-3-carboxylates, engaging these species in intermolecular coupling reactions under visible light irradiation. As a continuation of our program preparing F(sp^3)-rich, strucrually complex molecules for DNA-encoded library technology (DELT) applications via photocatalysis, we disclose herein the incorporation of unique and densely functionalized 2-oxa-1-azabicyclo[3.2.0]heptanes via [2+2] cycloaddition energy transfer sensitization, providing access to an unexplored library of azaspiro compounds, many of which include additional synthetic handles important for further functionalization of the DNA-conjugated products and for library production.

Introduction

DNA-encoded library technology (DELT) is considered a promising screening modality under continuous development in drug discovery programs, providing opportunities for the rapid, efficient, and cost-effective identification of potential drug candidates.[1]

DEL design begins with building block (BB) selection based on the potential of the final products to exhibit the most favourable drug-like properties. Recent developments in drug discovery have demonstrated that a high fraction of sp^3-hybridized carbon centers (Fsp^3)[2] is associated with greater clinical success in drug molecules by increasing potency, selectivity, solubility, and minimizing off-target effects.[3] Therefore, there is a continued interest in pursuing mild chemical transformations compatible with the sensitive nature of the DNA tags to incorporate diversity to the attached BBs.

Our efforts have been directed toward developing and translating robust photocatalytic transformations into on-DNA reactions and preparing structurally diverse and functionally rich DNA-encoded libraries as a platform for drug discovery research.[4-8] Given the mild conditions, these visible-light-mediated protocols have demonstrated superior results regarding the DNA-tag integrity, and this approach is thus one of the most reliable tools for DEL preparation.[1]

In the toolkit of medicinally prominent compounds, small, saturated rings have recognized relevance as effective drug targets given their physicochemical properties and role as bioisosteres.[9] Recently, we demonstrated a Giese addition to install highly functionalized bicyclo[1.1.1]pentanes (BCPs) on-DNA using tricyclo[1.1.1.0]pentane (TCP) as a radical linchpin.[8] Furthermore, we reported an efficient on-DNA [2+2] photocycloaddition reaction between heterocycles and exo-methylenecyclobutanes, leading to the preparation of novel and complex sp^3-rich spirocyclic compounds.[10] The unique structural nature of the products, the operational simplicity of the protocols, and the outstanding DNA integrity results, distinguishes this research program in its preparation of 3-D, F(sp^3)-rich building blocks on-DNA (**Figure 1a**). These strategies allow drug discovery teams to evaluate new sets of small, saturated compounds during hit identification on-DNA,[11] which may add the key properties required for the next new generation of therapeutics. Therefore, the growing of this screening modality field is driven by the advances in the synthetic protocols to prepare more diverse and elaborated libraries on-DNA. Recent research has shown that novel shapes and scaffolds in new molecular entities are more likely to have promise as unique therapeutic drugs in comparison to substructures already well represented in FDA-drugs, being one of the parameters for measuring pharmaceutical innovation.[12]

From this perspective, we have targeted the preparation of spatial, multi-oriented 2-oxa-1-azabicyclo[3.2.0]heptanes on-

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DNA. The target compounds, azaspiro[3.3]heptanes, in particular, have been shown to have significantly improved pharmacokinetic properties when compared to their amine analogues, and a growing number of patents have examples incorporating these sp^3-rich moieties as analogues of 3- and 4-substituted piperidines (**Figure 1b**).[13-16] Although considered a very promising modality,[17] there are only a few approved drugs containing azetidine-type rings, in large part because of the inherent lack of availability and challenges associated with their preparation.

Circumventing the use of high energy irradiation and the limitations associated with the scope of traditional aza-Paternò-Büchi reactions,[18] the Schindler group elegantly demonstrated that 2-isoxazoline-3-carboxylates could be engaged in an intermolecular, regioselective aza-[2+2] cycloaddition,[19] revolutionizing how azetidine-containing compounds can be accessed.[19-21]

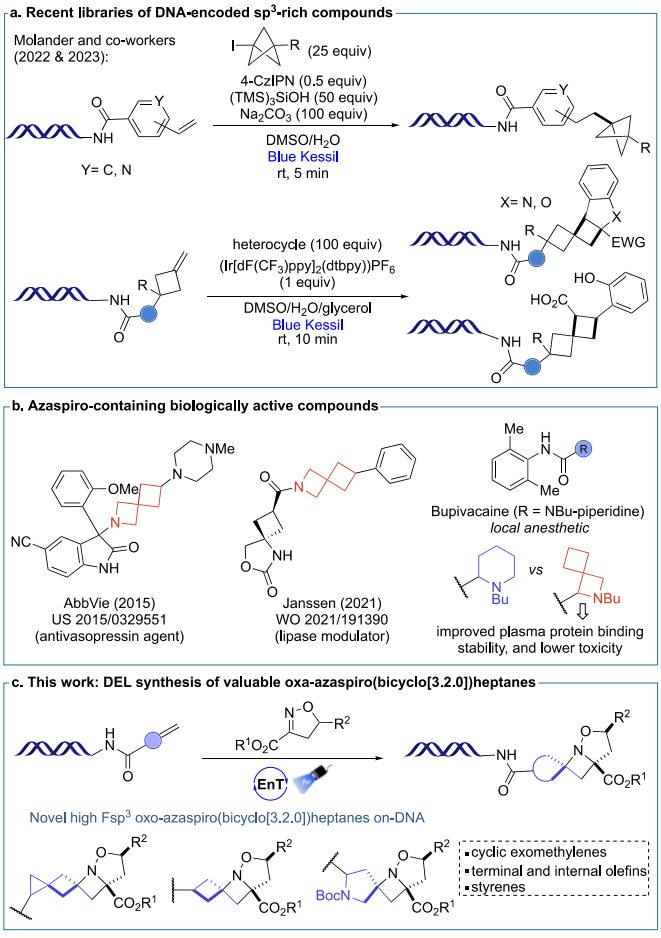


Figure 1. a. Recent work from our research group preparing sp^3-rich compounds on-DNA; b. Relevance of azaspiro-containing pharmaceutical compounds; c. This work - preparation of spatially multi-oriented 2-oxa-1-azaspiro(bicyclo[3.2.0])heptanes for DELT applications.

Inspired by these recent contributions and the emergent need to explore new libraries in DELT, we identified suitable conditions to access unique 2-oxa-1-azabicyclo[3.2.0]heptanes on DNA tags. Upon optimization studies (see Section 3 of SI for detailed information), we found that photo-inert exomethylene cyclobutane headpieces afforded valuable spirocyclic compounds after only 10 minutes reaction, under open-to-air conditions, when reacted with (hetero)aromatic-, alkyl-, and spirocyclic oxazolines in a very efficient manner, preparing unexplored sp^3-rich architectures (**Scheme 1, top**). 4-Substituted aromatic oxazolines smoothly underwent the desired transformation (**2**-**4**). Importantly, the azabicyclospiro adduct bearing a bromo group was successfully obtained (**3**), which could serve as a useful handle for further diversification. 5,5-Disubstituted substrates containing either phenyl- or ethyl groups also showed good reactivity toward the stepwise cycloaddition reaction, as in **5** (see SI for the entire library). Compound **6** with two ester groups gave the final adduct in excellent conversion. The decorated spirocyclic oxazoline **7** provided the even more elaborate cycloaddition adduct in high conversion, which is of special interest given the recognized properties of azaspirocyclic compounds modulating drug-like molecule properties.[12,13,22]

To incorporate BBs with the desired structural complexity to enrich DELs, we focused our attention on preparing other substituted azaspiro[3.3]heptane surrogate HPs.[10] Significant efforts have been made to prepare azaspiro[3.3]heptanes because of their utility in drug discovery (vide supra), particularly in the replacement of piperidine rings. Exomethylene cyclobutane HPs containing additional cyclopropane and cyclopropane/pyridine rings were therefore evaluated. Fortunately, they smoothly provided access to interesting and unique structures while exhibiting excellent reactivity in the presence of mono- and disubstituted oxazolines with aliphatic and aromatic groups at the 5-position (**8**-**13**). Other esters functionalized at the 3-position were also introduced, such as those with a 4-methoxybenzyl group (**9**), a propynyl synthetic handle (**10**), as well as the sterically hindered adamantyl group (**13**). This latter was also prepared on a 100-nmol scale with >95% conversion under standard reaction conditions. Additionally, compound **11** led to the corresponding carboxylic acid upon hydrolysis in the presence of sodium acetate and magnesium chloride to provide the final compound with a 50% conversion (**12**). These results further demonstrate the promising future applications of this synthetic modality on-DNA for DEL screening applications.

Completing the decorated molecules prepared in this library, an exomethylene containing an *N*-Boc-pyrrolidine group was also introduced to provide structurally relevant 4-oxa-5-azaspiro[bicyclo[3.2.0]heptane pyrrolidines that offer an additional site for further functionalization after *N*-Boc deprotection. 4-Aryl-substituted derivatives, such as 4-bromo- and 4-methoxy isoxazolines (**14** and **15**), provided the desired products in high conversions. The scope was also complemented by other important structural features for drug-like compounds, such as the presence of pyridin-2-yl (**16**) and pyrazidin-2-yl (**17**) heteroaromatic groups, and the *N*-Boc-azetidine spirocycle (**18**), furnishing additional sites for library derivatization and applications in DELT.

Next, we investigated additional alkene-containing headpieces, including terminal aliphatic alkenes, acrylamides, and internal olefins (**Scheme 1, center**). The headpiece (HP) derived from pentenoic acid furnished the cycloaddition products **19** in good

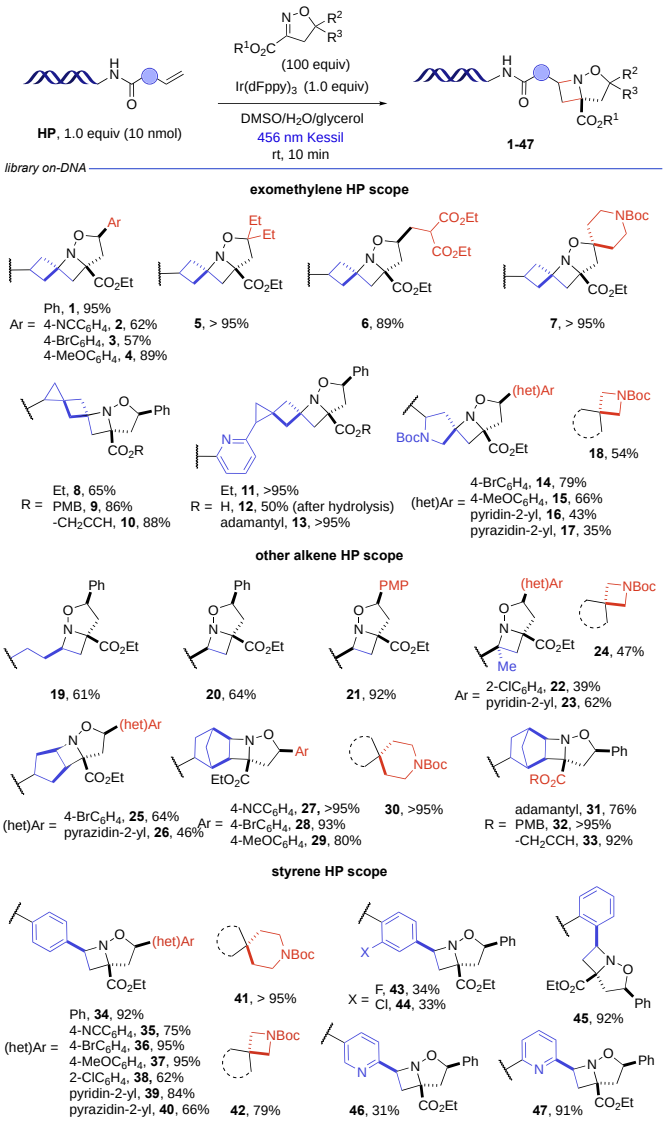
yield, while an acrylamide-type HP was also evaluated, furnishing **20** and **21**, in addition to the methacrylamide derivatives (**22-24**).

Overall, HPs containing internal olefins, such as those in the derivatives of cyclopentene (**25** and **26**), and bicyclo[2.2.1]heptyl carboxylates (**27**-**33**) also accommodated a range of substituted reaction partners. These included (hetero)aryl- and *N*-Boc-spiro-substituted isoxazolines in addition to the ones containing different ester groups (**31**–**33**). These examples present useful functional handles such as bromo- and alkynyl groups that offer opportunities for additional transformations.

Lastly, we investigated activated alkenes in styrene-type headpieces for preparing 3,7-diphenyl-2-oxa-1-azabicyclo[3.2.0]heptane derivatives (**Scheme 1, bottom**). The off-DNA transformation using styrene and 2-isoxazoline-3-carboxylates proved to be inefficient,[19] which could be attributed to photocatalyst quenching of the excited-state by this type of alkene, with excited state triplet energies ET≅ 60 kcal mol^{-1}.[23]

However, when a styrene headpiece was employed under the optimized conditions on-DNA, the reaction outcome was not impacted by the nature of the alkene attached on the DNA-tag, providing the corresponding adduct in the presence of the standard 5-phenyl-4,5-dihydroisoxazole-3-carboxylate with 92% conversion (**34**). The feasibility of the transformation on-DNA could be attributed to the use of a large excess of isoxazoline (100 equivalents), which would push the reactivity toward the cycloaddition event with the styrene-type HP. Other isoxazolines were also evaluated to expand the library of this class of compounds. Electron-rich and electron-poor 4-aryl-substituted groups at the 5-position of the isoxazoline successfully provided the desired adducts with pendant cyano-, methoxy-, and halogenated groups (**35**-**38**). Other variations of the isoxazoline partner included 5-(pyridin-2-yl) (**39**) and 5-(pyrazidin-2-yl) (**40**) substrates, as well as inclusion of *N*-Boc-spiro piperidine (**41**) and -azetidine (**42**) rings in excellent conversions. Both 3-chloro- and 3-fluoro-4-vinylbenzene headpieces also underwent the photocycloaddition reaction with synthetically useful conversions (**43** and **44**). A 2-vinylbenzamide headpiece also afforded the functionalized 3,7-diphenyl-2-oxa-1-azabicyclo[3.2.0]heptanes in excellent conversion (**45**). Next, we explored the scope of vinylpyridine headpieces. The activated 2-vinylpyridine showed lower reactivity toward the photocycloaddition with the triplet-state isoxazoline species in comparison to the more electron-neutral 2-vinylpyridine (**46** and **47**, respectively), which demonstrated excellent reactivity to provide the cycloadducts on-DNA.

With these results, the scope of the transformation demonstrated outstanding utility in the preparation of complex 2-oxa-1-azabicyclo[3.2.0]heptanes, comprising a rich array of F(sp^3)-rich DNA-tagged compounds. This protocol affords a new venue for medicinal chemists to investigate the therapeutic activities of novel shapes and structurally diverse classes of compounds that contain special properties regarding their structural composition.



Scheme 1. Scope of the on-DNA [2+2] intermolecular photocycloaddition reaction. Conditions: DNA (2 mM in H_2O, 10 nmol, 1.0 equiv), isoxazoline (100 mM in DMSO, 100 equiv), [Ir(dFppy)$_3$] (2 mM in DMSO, 1.0 equiv), glycerol (10 μL of a 20% solution in DMSO), rt, 10 min, blue Kessil. Work-up: Ethanol precipitation. PMB = *para*-methoxybenzyl; PMP = *para*-methoxyphenyl.

DNA Damage Assessment

An analysis of DNA integrity following a chemical transformation is a critical point of evaluation for new on-DNA chemistries. To understand the impact of the developed aza-[2+2] cycloaddition conditions on DNA, an elongated 50 bp DNA tag bearing an *exo*-methylene cyclobutylamide functional group was prepared and subjected to the developed photocycloaddition conditions. Following photocycloaddition, the spirocyclic azetidine products were shown to maintain high efficiency in ligation reactions (94% yield by LCMS). qPCR analysis of the spirocyclic azetidine products demonstrated 74% amplification efficiency, which is identical to that of control reactions in the absence of photocatalyst, light, or without being subject to the reaction conditions. Finally, next generation sequencing (NGS) analysis revealed only 1% mutated sequences following the on-DNA photocycloaddition. The summation of these data show that the developed reaction

is compatible with the DNA tag backbone and, therefore, of valuable interest for applications toward new library production.

Conclusion

Continuing our research program in the preparation of valuable drug-like small molecules on-DNA, we present herein an efficient preparation of structurally complex, F(sp^3)-rich 2-oxa-1-azaspiro(bicyclo[3.2.0])heptanes on-DNA, harnessing the reactivity of the 2-isoxazoline-3-carboxylate excited-states with a diverse range of alkene DNA-headpieces. DELT is an impactful screening modality that will benefit from advances in organic synthetic methods that are designed to be compatible with the sensitive nature of the DNA tag. In this regard, the visible light-mediated protocol demonstrated herein has the potential to provide access to important spirocyclic compounds for drug discovery. The reaction also allowed expansion to styrene-type acceptors, which previously demonstrated poor reactivity toward [2+2] photocycloaddition with 2-isoxazoline-3-carboxylates in the off-DNA transformation. Evaluation of the DNA integrity demonstrated the developed conditions are compatible with the DNA backbone, as the post-reaction DNA material maintained high ligation efficiency and qPCR amplification efficiency, with only 1% of base pair mutations as detected by NGS analysis. With this work, we were successful in accessing new F(sp^3)-rich chemical space to increase molecular complexity in DELT. We believe this chemistry has the potential to stimulate additional new advances in application of photo-redox catalysis to the DELT field.

Author Contributions

B.M. developed the reported transformations, conducted and analysed experiments. S.K., S.S., and G.L. prepared starting materials. L.L. prepared some of the headpieces used in the study and helped with reaction optimization in the early stage. The manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

Conflicts of interest

The authors declare the following competing financial interest(s): Some authors are employees of AbbVie. The design, study, execution, and financial support for this research were provided by AbbVie and the agencies noted below. AbbVie participated in the interpretation of data, review, DNA damage assessment, and approval of the publication. EAC and EJM are former employees of AbbVie.

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