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PHARMARON

Alkyne Iodination and Acetylenic Couplings on-DNA

A new tool to access uncharted chemical-space in DEL Synthesis

Pharmaron is a premier R&D service provider for the life sciences industry that offers a broad spectrum of research, development and manufacturing service capabilities throughout the entire drug discovery, preclinical and clinical development process across multiple therapeutic modalities, including small molecules, biologics and CGT products.

Introduction

Iodoalkynes and diynes are particularly relevant medicinal molecules that can both act as precursors and active compounds. While a variety of approved molecules contain these uncommon functionalities, it is their strained structure that makes them attractive as far as chemical space is concerned.[1-6] Although, a wide array of classical synthetic pathways has been unveiled to access them, they were both proved to be particularly challenging to access on-DNA and are consequently missing from DEL libraries. Pharmaron's team set course on the development of DNA-compatible methodologies to efficiently yield both iodoalkynes and diynes by using mild and DNA-safe copper-mediated:

- **i) iodination of terminal alkynes**
- **ii) acetylenic couplings of these aforementioned products[7]**

This strategy offers the possibility to rapidly include novel structures and chemical space properties in DEL libraries.

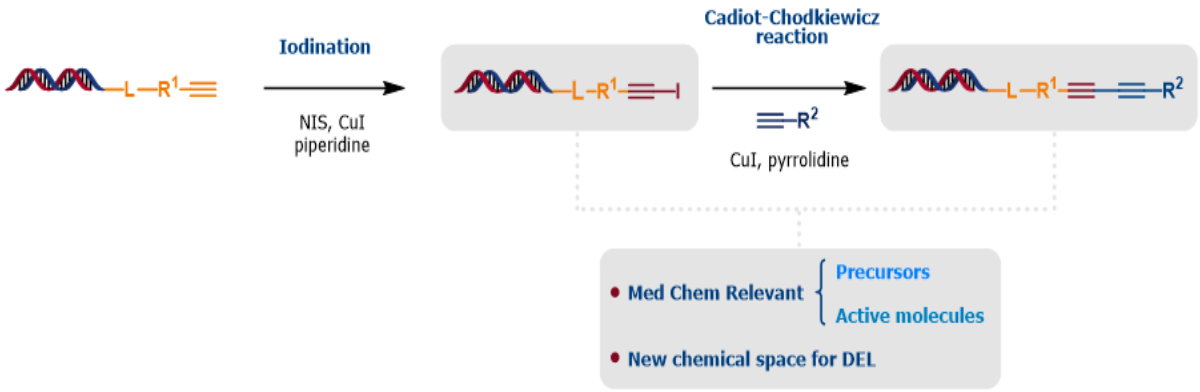
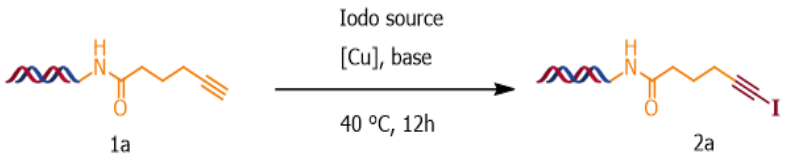


Fig. 1: On-DNA iodination and Cadiot-Chodkiewicz reaction of alkynes.

On-DNA Alkyne Iodination: Development and Scope of the Reaction

The iodination reaction allows for the transformation of a DNA conjugated terminal alkyne into the corresponding iodoalkynes. Despite the known sensitivity of oligonucleotides to oxidative media, a careful screening of conditions demonstrated the possibility to conduct this transformation efficiently without endangering the encoding DNA tag. Table 1 shows that the desired model DNA conjugated iodoalkyne can be obtained in high yields by using excess of copper iodide, NIS (*N*-iodosuccinimide) as the iodine source and piperidine as the base. Optimal conditions are achieved upon using the conditions highlighted in Table 1.



Entry	[Cu] (eq.)	[I] (eq.)	base (eq.)	2a (%)[a]
1	CuI (10)	-	Piperidine (100)	12
2	CuI (10)	KI (20)	Piperidine (100)	29
3	CuI (10)	NIS (20)	Piperidine (100)	87
4	CuBr (10)	NIS (20)	Piperidine (100)	86
5	$Cu(MeCN)_4PF_6$ (10.0)	NIS (20)	Piperidine (100)	86
6	CuI (10)	NIS (20)	Cy_2NMe (100)	31
7	**CuI (5)**	**NIS (20)**	**Piperidine (50)**	**95**

[a] conversion determined by LC-MS

Table 1: Optimization of the reaction conditions – selected screening entries.

Selected examples of the reaction applicability are showcased in Table 2.[7] Typically, most alkynes substrates allow good conversion. Aliphatic and aromatic alkynes with an amide link to the DNA tag react particularly well (2a to 2k). Steric hindrance can influence the outcome of the iodination reaction as illustrated by the ortho-substituted 2g. The iodination is also well tolerated for substrates having a reverse amide link to DNA (2k to 2m). Heterocyclic substrates such as thiophene and pyridine-alkyne substrates can be efficiently reacted (2o and 2p).

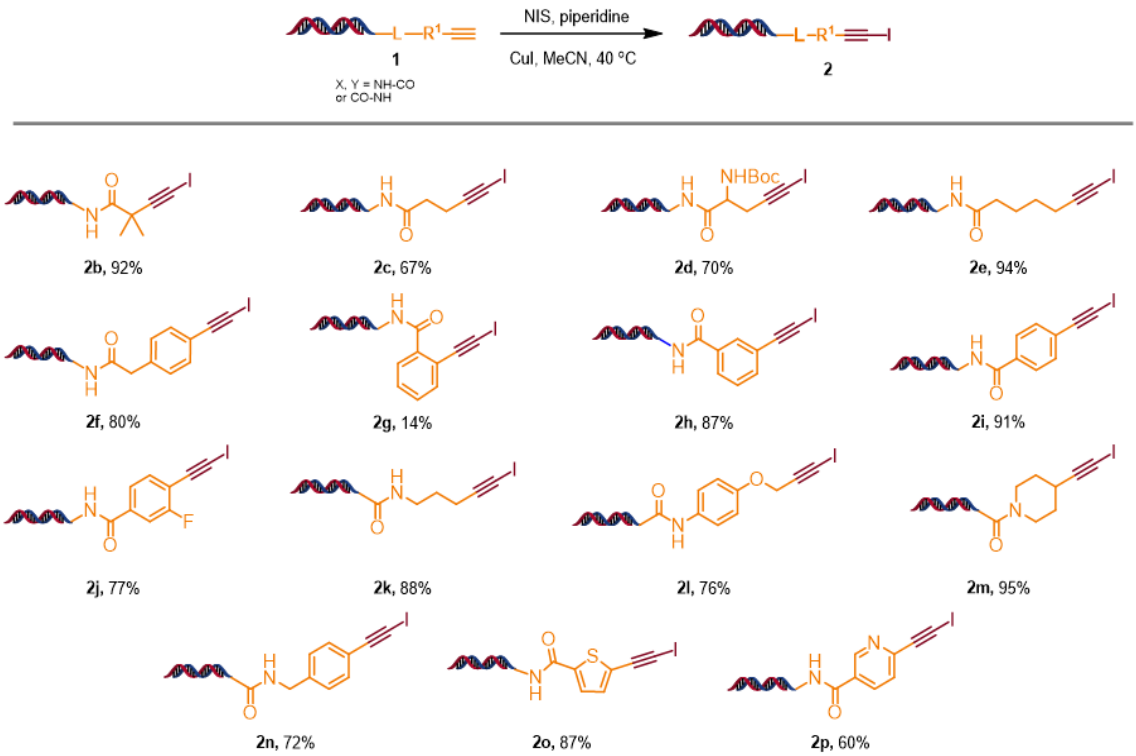
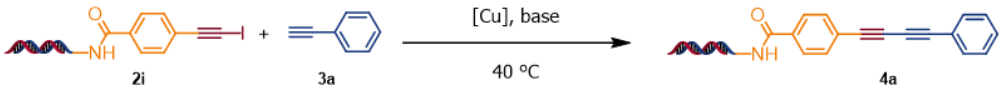


Table 2: On-DNA alkyne iodination – selected examples.

Acetylenic Coupling Via Cadiot-Chodkiewicz Reaction: Development and Scope

While iodoalkynes can be considered as highly relevant medicinal compounds, they also represent valuable precursors as they are versatile platforms for homologation reactions to a variety of building blocks.[1-3] Given the lack of precedent regarding the formation of 1,3-dialkyne motifs, the development of an efficient pathway to access these scaffolds is particularly desired. Cadiot-Chodkiewicz coupling,[1] involving a copper catalyst seems particularly appropriate to transform an iodoalkyne and an additional terminal alkyne into corresponding dialkyne product. Our optimization study (Table 3) demonstrated that this coupling can be carried out using reaction conditions similar to the ones employed for the iodination reaction. Optimal conditions were found to consist in copper iodide as the catalyst, pyrrolidine as the base and the terminal alkyne partner in large excess, allowing almost full conversion to the target compounds.



Entry	[Cu] (eq.)	base (eq.)	3a (eq.)	4a (%)[a]
1	CuI (20)	Pyrrolidine (200)	200	52
2	CuI (10)	Cy_2NH (100)	200	30
3	CuI (10)	Cy_2NMe (100)	200	10
4	CuI (10)	Piperidine (100)	200	45
5	CuBr (10)	Pyrrolidine (200)	200	30
6	$Cu(CF_3SO_3)_2$ (10)	Pyrrolidine (200)	200	10
7	CuI (10)	Pyrrolidine (200)	400	84
8	**CuI (10)**	**Pyrrolidine (400)**	**400**	**95[b]**

[a] conversion determined by LC-MS, [b]Reaction ran for 24 hours

Table 3: Optimization of the reaction conditions – selected screening entries.

Table 4 contains several selected examples of the Cadiot-Chodkiewicz reaction using the optimal conditions.[7] The reaction performs particularly well upon using aromatic or heteroaromatic acetylenic substrates and the same trend was observed upon using aliphatic ones, whatever the functionalizing pattern present. However, some examples prove that copper chelation can disrupt the catalytic cycle and lead to lower conversions when heterocyclic substrates are present (4k and 4l). As suggested, both transformations (on-DNA alkyne iodination and coupling) can be conducted in a one-pot two-step process as the conversion of 4a reached 85% for 2 consecutive steps when a precipitation step was employed between each reaction (compared to 73% for a stepwise method).[7]

Application to DNA-encoded Libraries Syntheses

A conceptual dialkyne 182-member DEL design is described in Figure 2, consisting of two cycles. Unique coding sequences relevant to each component are also ligated and the library was built using 9 carboxyl acid-alkynes and 5 amine-alkynes as Cycle 1 building blocks and 13 alkynes as Cycle-2 reagents, and final UPLC purification. Using real-time qPCR, we can confirm that these two transformations, do not alter DNA tags integrity, despite being conducted sequentially. The DNA tag not containing reactive functionalities was also engaged in the optimal conditions and results confirmed unaltered structure.

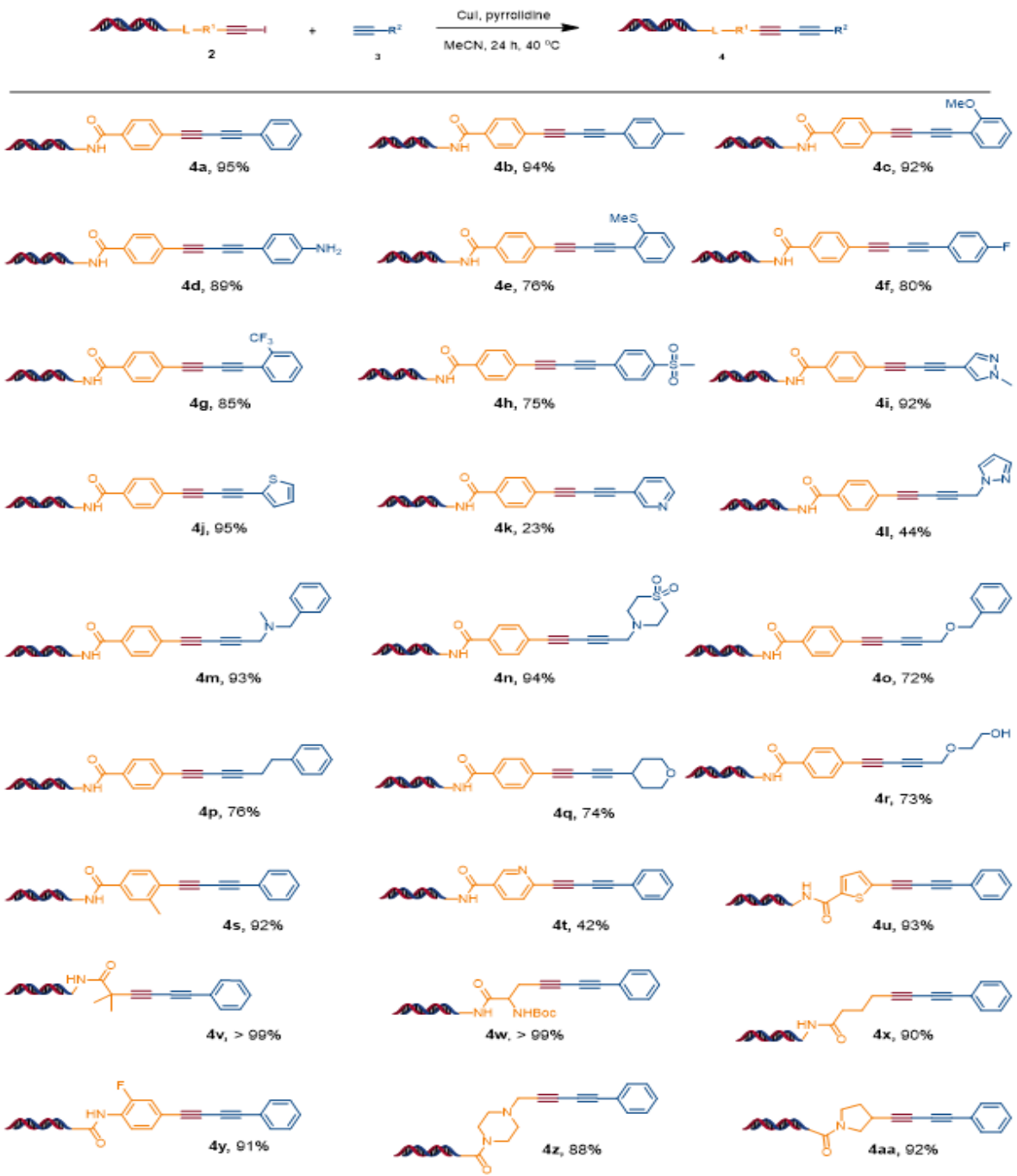


Table 4: On-DNA Cadiot-Chodkiewicz reaction – selected examples.

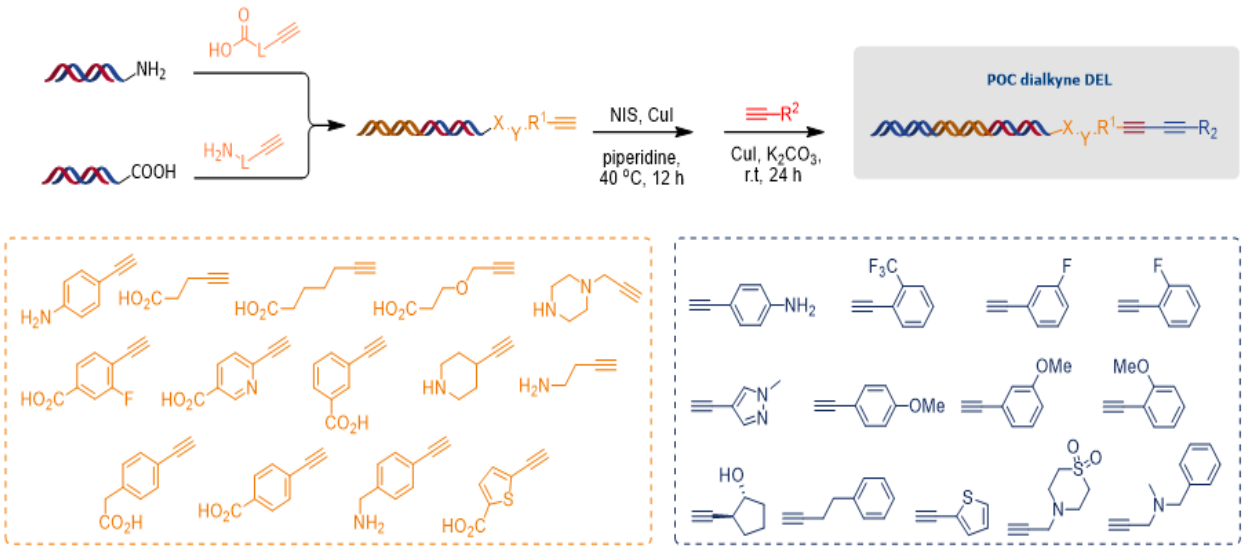


Fig. 2: Prototypical dialkyne DEL design and synthesis.

Key Takeaways

Pharmaron's novel methodology routinized the generation on DNA of iodoalkyne and 1,3-dialkyne for DEL synthesis granting the following advantages:

1. Direct access to 2 classes of highly relevant precursors and medicinal chemistry relevant cores
2. Rapid generation of unprecedented DEL compounds allowing drastic enrichment of the chemical space
3. DNA-safe process which can be conducted in a one-pot two-step process

References:

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