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Retinoids Molecular Probes by Late-stage Azide Insertion – Functional Tools to Decrypt Retinoid Metabolism

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Studying the complex and intricate retinoids metabolic pathways by chemical biology approaches requires design and synthesis of biologically functional molecular probes. Only few of such molecular retinoid probes could be found in literature, most of them bearing a molecular structure quite different from natural retinoids. To provide close-to-native retinoid probes, we have developed a versatile late-stage method for the insertion of azide function at the C4 position of several retinoids. This

one-step process opens straightforward access to different retinoid and carotenoid probes from commercially available precursors. We have further demonstrated that the different molecular probes retain ability of the original compound to activate genes' transcription, despite azide insertion, highlighting biological activities that were further validated in zebrafish *in vivo* model. The present work paves the way to future studies on vitamin A's metabolism.

Introduction

Vitamin A1, frequently called vitamin A, is defined as a group of physiological retinoids whose mainly active form is all-*trans* retinoic acid (*atRA*).^[1,2] Vitamin A is acquired in nutrition, most frequently in the form of retinyl esters, present in products of animal origin and in particular meat or offal of ruminating animals. Thus, retinyl esters are mostly detected in blood during the postprandial period before they reach their storage sites, mainly the liver, but also other organs and tissues, notably adipose tissue.^[3] The hydrolysis of retinyl esters by retinyl ester hydrolases such as the pancreatic triglyceride lipase (PTL), carboxyl ester lipase (CEL) and lipoprotein lipase (LPL) or hormone-sensitive lipase (HSL)^[4,5] generates all-*trans* retinol (*atROL*) and ensure its steady levels in this circulation and delivery to target cells throughout the organism.^[6] In target

cells, *atROL* is transformed into *atRA* by two successive oxidative steps, first to all-*trans* retinaldehyde (*atRAL*), through a reversible reaction catalyzed by retinol dehydrogenase / retinaldehyde reductase complex, and then to *atRA* following an irreversible oxidation catalyzed by retinaldehyde dehydrogenases.^[7] Due to their good stability and low toxicity, retinyl esters are the preferred vectors for vitamin A supplementation strategies in clinics or cosmetics. An alternative nutritional source of vitamin A, mainly found in fruits and vegetables, is α - or β -carotene (β C), the latter leading to two molecules of *atRAL* upon oxidative cleavage by β -carotene-oxygenase (BCO1)^[8] which can then be transformed to either *atROL* for esterification and storage or to *atRA*.^[9] In addition, new bioactive metabolites or types of vitamin A continue to be discovered, including the vitamin A metabolite 4-oxo RA,^[10] or 9-*cis*-13,14-dihydro RA, an active form of a new class of vitamin A, called vitamin A5/X.^[11] Thus, we cannot exclude that other forms of active retinoids have yet to be discovered but their identification is currently hampered by technological limitations.

atRA was reported as essential for a wide variety of cellular functions ranging from cell proliferation and differentiation^[12,13] to apoptosis.^[14,15] Vitamin A controls also a number of physiological processes including vision,^[16] maintenance of immunity^[17] and brain functions.^[12] Such activities are classically mediated by binding and controlling retinoic acid receptors (RAR α , RAR β , RAR γ) which act as ligand-regulated transcription factors that control transcription of a wide variety of target genes.^[18] Differential expression of RARs, but also retinoid binding proteins and metabolic enzymes is essential for precise control of retinoid signaling and underlays pleiotropic actions of vitamin A. Accordingly, an excess or deficit in *atRA* availability leads to congenital malformations including respectively teratogenicity or vitamin A deficiency (VAD) syndrome. A number of neurodegenerative diseases were also associated with compromised retinoid signaling due to reduced expression of *atRA* synthesizing enzymes or receptors in Alzheimer's,^[19]

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Parkinson's,^[20] amyotrophic lateral sclerosis^[21] or Huntington disease.^[13] These observations indicate that retinoids have great medical potential in treatment of neurologic disorders.^[22–24] Although vitamin A signaling has been very widely studied, the existing knowledge does not allow its full understanding. For example, much of the knowledge on the storage, traffic and metabolism of retinoids is limited to intestine, liver or adipose tissue, but much less is known about other tissues also known to contribute to these processes.^[6] This may result, at least partially, from technological limitations in the sensitivity of retinoids' detection and analyses due to their low physiological levels coupled with difficult extraction and separation or poor ionization. In this context, chemical biology approaches relying on molecular probes and metabolic labeling might increase the sensitivity of the detection method or provide alternative analytical strategies to decipher this complex metabolic network and shed light on the dynamic aspects of retinoids' interconversion. Such approaches require design, synthesis and testing of retinoid derivatives, which can enter metabolic pathway of vitamin A, and which are tailored to facilitate their detection. Surprisingly, only few retinoid-based molecular probes could be found in literature, most of them bearing a molecular structure quite different from natural retinoids.

As example, Khatib *et al.* have published *bis*-aryl-based compounds^[25] exhibiting ability to bind RARs, leading to the transcription of corresponding genes (Figure 1, A). In the absence of the characteristic isoprenoid chain, the physico-chemical properties of such derivatives are too divergent from endogenous retinoids, hence compromising their potential metabolization by retinoid converting enzymes and impeding relevant chemical biology studies. Chisholm *et al.*, and Tomlinson *et al.* have also reported fluorescent *bis*-aryl-alkyne for cell imaging.^[26] But again the molecular structure is quite different from both endogenous retinoids and late generations of synthetic retinoids (Figure 1, B and C). On the other hand, Bos *et al.* succeeded in synthesizing probes structurally related to natural *atRAL* (Figure 1, D), bearing a modification on the C3 position of the cyclohexene ring, and used them to study retinoids uptake in human immune cells.^[27,28] This strategy, while promising in the context of a metabolomics study, is hampered by the need to perform a eleven-step total synthesis,

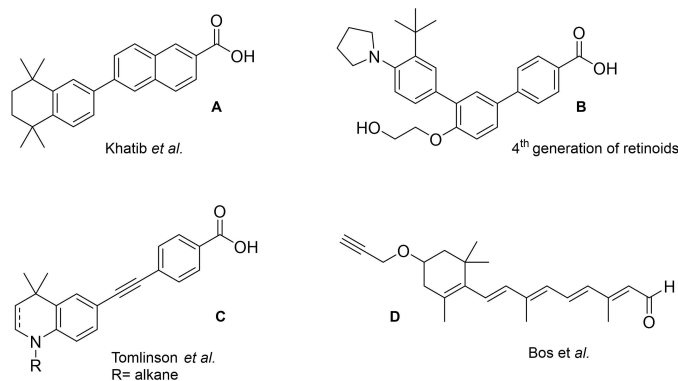


Figure 1. Different synthetic retinoids described in the literature.

involving delicate and unstable intermediates, affording the title compound in less than 10% overall yield.

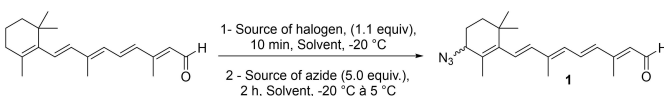
To overcome these drawbacks and open access to larger panel of molecular probes closely related to native retinoids, we searched for a mild and quick late-stage functionalization approach. (Scheme 1) Not only should this approach provide probes in a single step from readily available retinoids, it should also be suitable for bio-orthogonal applications. In this context, we focused on azide incorporation: thanks to its small size, such motif should minimize the risks of altering the retinoids' biological activity,^[29] whilst being poised for Strain-Promoted Azide Alkyne Cycloaddition (SPAAC) for further modification with cycloalkynes bearing an extended library of imaging,^[30] chemo-proteomic,^[31] or MS tags.^[31,32]

In the literature, diverse strategies are described for late-stage insertion of azide functions. Nonetheless, none of them are compatible with either the retinoids' fragile structure or current environmental concerns. Indeed, the majority of late stage azidations is only applicable to chemical functionalities absent from retinoids (e.g., 1,3-dicarbonyles,^[33] aliphatic substrate,^[34] glycals)^[35] and/or use metal catalysts (e.g., iron,^[36] copper,^[37] manganese)^[38] that might lead to side reactions with the retinoids polyene chain. However, we noticed the papers of Anil Kumar Singh,^[39] in 1983, that was taken up in 2007 by Hashimoto^[40] and described the preparation of retinal oxygenated derivatives at C4 position through the activation of retinal with NBS. This assumption confirmed the report of Sheves *et al.* in 1984,^[41] who suggested the formation of a photoreactive azido-retinal intermediate upon addition of an excess of sodium azide post NBS activation. Even though the mechanism remained elusive and retinal derivatives poorly characterized, it appeared to us that optimizing this reaction could lead to a clean activation process enabling the introduction of a nucleophilic azide. But the first concern was to know at which position this azido insertion should occur on retinoids, to be easily synthesized chemically without disturbing biological activity of origin.

Results and Discussion

Structural Biology Guided Strategy to Identify the Best Position for Azido Insertion on Natural Retinoids

Docking studies were performed using SwissDock server and RAR α Ligand Binding Domain (LBD) crystal structures as target. The *atRA* was first re-docked into the RAR α LBD and the docking reproduced the co-crystallized ligand pose (PDB 3A9E). The best binding models for the other docked ligands, *atROL*, *N₃-atROL*, *atRAL*, *N₃-atRAL* and *N₃-atRA*, correspond to the



Scheme 1. Reaction of azidation on the C4 position of *atRAL*.

ligands bound into the canonical binding pocket in poses similar to that adopted by the reference ligand with the β -ionone ring closed to H11-H12 lid and H5 lying above the isoprene tail (Figure 2).

The computed binding energies of the *atROL* and *atRAL* (Figure 2C) are lower than that of *atRA* that makes more interactions via its carboxylates. To accommodate the azido group, the β -ionone ring is shifted by 1.5 Å in *N₃-atRA* / *RAR α* model or rotated in *N₃-atROL* and *N₃-atRAL* models (Figure 2B). The differences in the computed binding energies of the azido modified ligands seem to derive from the stronger additional interactions formed by the azido group (Figure 2A). Indeed, while preserving the interactions of *atRA*, the azido group of *N₃-atRA* forms stronger interactions with W225, V395, L398, M406 and I410 (Figure 2A).

These structural results allowed us to select the C4 position for azido insertion, without the latter interfering with binding to its nuclear receptor.

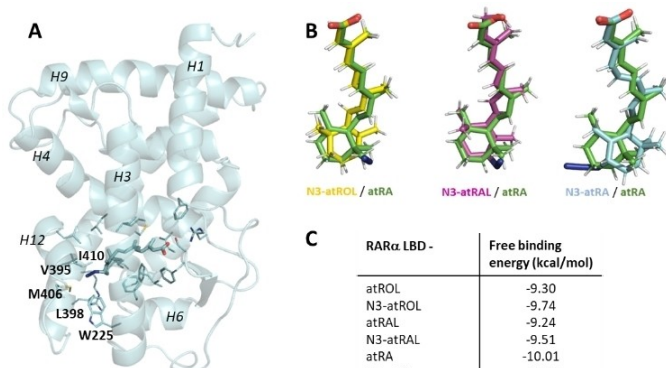


Figure 2. Models of the binding mode of the azido compounds to *RAR α* LBD. (A) Overall 3D model of *RAR-N3atRA* complex shown in cartoon representation. Labeled amino acids correspond to the residues interacting with *N₃-atRA* at a 4.0 Å cutoff. (B) Overlay of the docked poses of *atRA* with *N₃-atROL* (left), *N₃-atRAL* (middle) and *N₃-atRA* (right). (C) Free binding energy of the best models for the tested ligand-*RAR α* complexes. The crystal structures *hRAR* LBD-*atRA* (PDB 3 A9E) and *hRAR* LBD-AM580 (PDB 3KMR) were used as target for the docking that was performed using the EA Dock DSS docking software (<http://www.swissdock.ch/>) through the SwissDock server. Docking poses were visualized and structural figures were prepared using PyMOL.

Table 1. Screening of conditions for azide insertion on *atRAL*. Conversion was followed by analytical HPLC at 370 nm.

Entry	Halogen source	Azide source	Solvent	Conversion (%)
1	NBS	NaN ₃	CCl ₄	0
2	NBS	NaN ₃	CH ₂ Cl ₂	100
3	NBS	TBAN ₃	CH ₂ Cl ₂	100
4	NIS	TBAN ₃	CH ₂ Cl ₂	0
5	NCS	TBAN ₃	CH ₂ Cl ₂	0
6	TBATB	TBAN ₃	CH ₂ Cl ₂	100
7	DBDMH	TBAN ₃	CH ₂ Cl ₂	Degradation

Retinal

Considering the original activation conditions mentioned above, we subjected *atRAL* to conditions extrapolated from Sheves' report: *atRAL* was first reacted with NBS in CCl₄ for 10 min at -20 °C before sodium azide was added and the reaction stirred for 2 hours (Table 1, entry 1). However, to our dismay, no conversion of *atRAL* was observed and varying the reaction time or temperature led to no noticeable change.

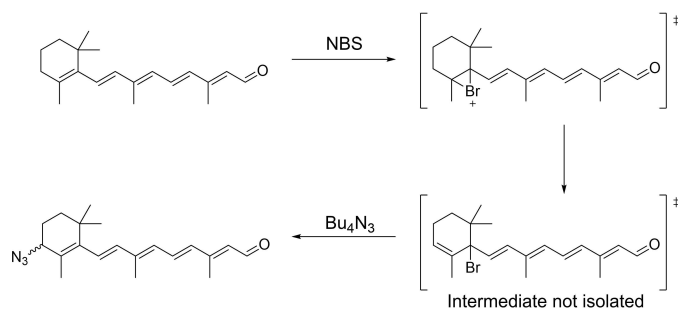
As the first step of the reaction was limiting, we suspected a lack of solubility of the bromine source. To overcome this problem, and regarding the toxicity of CCl₄, we replaced it with DCM (Table 1, entry 2). This resulted in 100% conversion of *atRAL*, confirming our hypothesis. However, a mixture between 1 and an unidentified by-product was obtained (60/40). We therefore continued the screening by changing the azide source (Table 1, entry 3), which led to 100% conversion in azido retinal 1. Entry 3 conditions were therefore considered optimal. We found that the reaction gives a mixture of isomers. According to proton NMR, in particular the proton in α of the azide function, the mixture consists of >85% all-*trans* azido retinal. The other isomer identified as 11-*cis* isomer constituted the major impurity (see Figure S11). Finally, we examined the effect of the electrophilic agent (Table 1, entries 4 to 7). With iodine and chlorine there was no reaction, probably due to the largest size of iodine and the lowest reactivity of chlorine. Changing the type of brominating agent, we observed the same result with TBATB, but a strong degradation of retinal with DBDMH, possibly due to the ability of this molecule to brominate olefins.^[42]

Proposed Mechanism

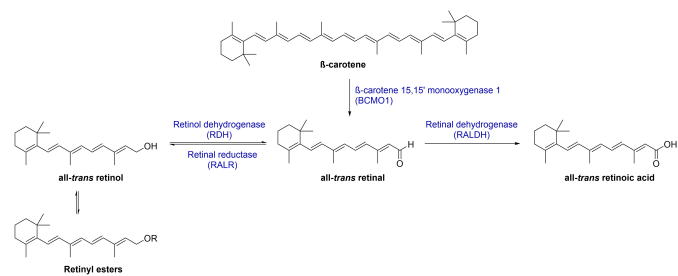
NBS is known to react *via* either ionic, bromonium intermediates or *via* radical species through Wohl-Ziegler reaction. In the latter case, a radical initiator is needed for the reaction to proceed – e.g., light, heat, microwaves, radical initiator.^[43,44] We thus ran our reaction under strict absence of radical promoter, in degassed solvent and in a dark room, and noticed that the yield of the transformation was unaffected, strongly ruling out a radical mechanism and rather advocating for a bromonium-type activation of *atRAL*. We thus hypothesize that the C4 activation proceeds by the preliminary formation of a bromonium ion from the most electron-rich alkene of the polyene chain (Scheme 2). Bromonium ring-opening through a formal E1 reaction would lead to brominated species that would undergo either a S_N2' reaction or a S_N1-Winstein rearrangement cascade with the azide salt to deliver the final product.^[45]

β -Carotene, Retinyl Acetate and Methyl Retinoate Azides

Having developed an efficient azidation strategy of *atRAL*, we then sought to evaluate its application to other retinoids (Scheme 3), namely β -carotene (β C), retinol (ROL) and retinoic acid (RA). As β C bears two C4 positions, the amount of



Scheme 2. Proposed mechanism for the azide insertion on position C4.



Scheme 3. Metabolic cascade of the known forms of vitamin A.

equivalent of reagents was doubled and cleanly delivered diazido β -C in 94% isolated yield (2). However, neither ROL nor RA gave the expected azido derivatives, presumably due to their free alcohol and carboxylic acid moieties, respectively, urging us to work with ester derivatives instead. Gratifyingly, both retinyl acetate and methyl retinoate led to their desired C4 azidated products 3 and 5, respectively, in 21 and 57% yield. In all three cases, the same degree of isomerization was observed, with about 90% of all-*trans* isomer being detected by ^1H NMR spectroscopy.

Azido Derivatives of *atRAL*, *atROLAc* and *atRAMe* are Biologically Active

To evaluate the impact of the C4 modification on the biological activity of our azido-retinoids, we tested their efficiency to transactivate retinoic acid receptors (RARs) in transcriptional reporter system in cultured cells. To this end, HEK 293T cells were transfected with GAL-RAR α together with 17mx5-G-Luc firefly luciferase and *Renilla-Glo* reporter plasmids as previously described.^[46] Comparison of specific retinoids with their azide-modified counterparts was performed by incubation of increasing concentrations of individual compounds over 5 hours with cells and followed by quantification of firefly luciferase activity and standardized for *Renilla-Glo* signal (GloMax®-Multi Detection System). Treatment with *atRA* was used as a positive control throughout the study.

As expected, *atRA* activated nuclear receptor RAR α even at the lowest concentration of 10^{-9} M (Figure 3). Instead, for the same duration of treatment *atRAL* displayed transcriptional activity only at 10^{-6} M, which may be explained by the necessity

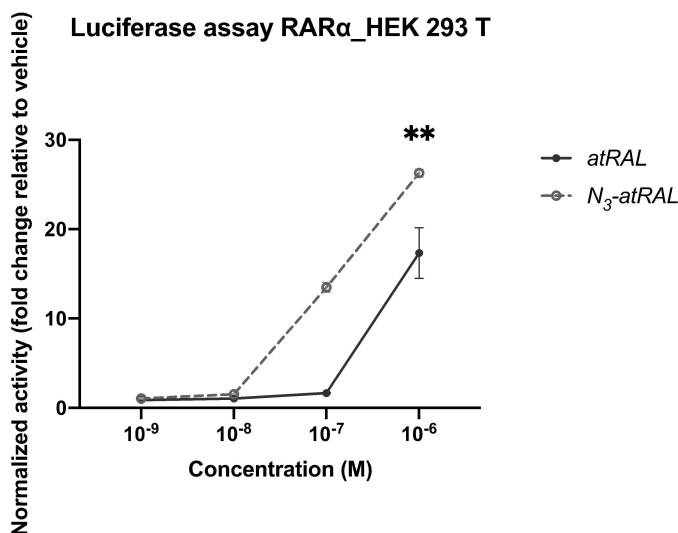


Figure 3. Luciferase assay with *atRA*, *atRAL* and *N₃-atRAL* activation of RAR α in HEK 293T cells depending on their increased concentrations (ranging from 10^{-9} M to 10^{-6} M). ** $p < 0.01$ as compared to *atRAL*. Results are expressed as means of $n = 3$ independent samples \pm SEM. All experiments were carried out in triplicates.

of enzymatic conversion of *atRAL* into bioactive *atRA* required for RAR transactivation. As revealed by two-way repeated measures ANOVA there was a significant difference between *atRAL* and *N₃-atRAL* on reporter expression with their increasing concentrations ($F_{(3,6)} = 258.8$, $p < 0.0001$ for main effect of the concentration). Accordingly, transcriptional activity evolved differently across different concentrations depending on treatment as documented by significant interaction between treatment and its concentration ($F_{(3,6)} = 12.1$, $p < 0.01$). As indicated by Bonferroni post-hoc analyses, this difference reflected higher transcriptional activity of *N₃-atRAL* with respect to *atRAL* which was evident already at 10^{-7} M ($p < 0.01$; Figure 3) as compared to *atRAL* which displayed similar activity only at 10^{-6} M. Thus, these data indicate that the insertion of the azido group in position C4 is compatible with transcriptional activity of retinoids and may even boost such activity in agreement with modeling data which predicted stronger binding to RAR α LBD (Figure 2). In conclusion we suggest that *N₃-atRAL* is suitable for biological studies of retinoid pathway.

Although *atRAL* is a direct precursor of the *atRA* *in vivo*, its clinical use was hampered in particular due to limited stability. In contrast, retinoid esters display good stability and low toxicity. For example, retinyl esters are some of the best documented *atRA* prodrugs and the acetate ester of ROL, *atROLAc* (retinyl acetate), was already studied as a potential inhibitor of certain tumor cell lines proliferation, e.g., C1300 neuroblastoma;^[47] but also, as a treatment to improve visual functions of mice lacking retinal pigment epithelium^[48] or for partial restoration of vitamin A deficiency in hemiparkinsonian rats.^[49] In line with these data, we hypothesized that azide-retinyl acetate could be a good precursor of bioactive azide-retinoids and thus display similar activity to *atROLAc*. To address this possibility, we compared the potential of *atROLAc* and its azido form, referred to as *N₃-atROLAc*, to induce the

transcription in luciferase reporter assay (Figure 4). As revealed by one-way ANOVA ($F_{(3,8)}=180$; $p<0.001$) and post-hoc Dunnett's test both esters applied at 10^{-6} M significantly activated transcription as compared to vehicle ($p<0.05$ and $p<0.001$ for and N_3 -*atROLAc* respectively). The activity of *atROLAc* was however significantly lower as compared to *atRA* ($p<0.05$; Figure 4), but also when compared to *atRAL* ($p<0.05$; see activity at 10^{-6} M in Figure 3). This result was not unexpected since *atROLAc* requires three enzymatic steps to be converted into the major, transcriptionally active form, *atRA*. Importantly, N_3 -*atROLAc* was at least twice more efficient as compared to *atROLAc* (Figure 4, $p<0.05$), which was reminiscent of higher activity of N_3 -*atRAL* as compared to its non-azidated form *atRAL* (see Figure 3).

Considering biocompatibility of C4 modifications in N_3 -*atRAL* and N_3 -*atROLAc*, we generated also a methyl ester carrying azide on C4 position, named here after N_3 -*atRAME*, as potential direct precursor of azido-retinoic acid, N_3 -*atRA*. As supported by one-way ANOVA analysis ($F_{(4,10)}=265.7$, $p<0.001$) and Dunnett's post-hoc comparison only *atRA* ($p<0.001$) and N_3 -*atRAME* ($p<0.001$) esters displayed significant activation of transcription as compared to vehicle. Absence of *atRAME* activity was surprising considering that only one step of enzymatic conversion is required to generate *atRA* from this precursor. This may reflect low expression of enzyme specific for retinoic acid esters in HEK 293T cells. In contrast, azido-retinoid counterpart N_3 -*atRAME* displayed some activity which

was consequently higher than that scored for *atRAME* ($p<0.001$ as compared to *atRAME*; Figure 5) and which reflects most probably higher affinity and activity of azido-retinoids. In line with low abundance/activity of retinoic acid esterase(s), transactivation by N_3 -*atRAME* was three times lower than *atRA* ($p<0.001$) but also lower than N_3 -*atROLAc*, the latter requiring three steps of conversion but most probably involving different enzymes (compare Figures 4 and 5).

In Vivo Evaluation of the Azido Derivative of *atRAL* as a Molecular Probe in a Zebrafish model

The above-mentioned results demonstrated that azido-retinoids display an improved *in vitro* activity as compared to the unmodified parent retinoids. We then aimed at determining the suitability of these retinoid derivatives as molecular probes in complex biological environments. First of all, we verified the ability of these azide-modified molecules to be functionalized through a SPAAC reaction in a biorthogonal manner. To this end, N_3 -*atRAL* was first diluted at a $10\ \mu\text{M}$ concentration in acetonitrile in the presence of 2 equivalents of DBCO-SulfoCy5. This straightforward reaction in an organic solvent served as a reference analyte for comparison when carried out in more complex media. The reaction was then repeated under the same conditions in murine and human plasma. In both cases

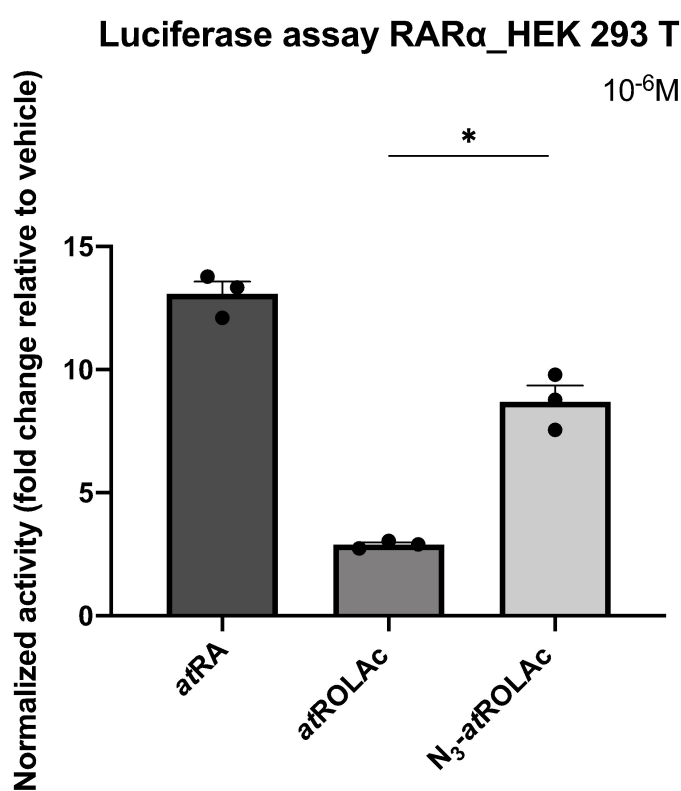


Figure 4. Luciferase assay at 10^{-6} M *atRA*, *atROLAc* and N_3 -*atROLAc* for activation of $RAR\alpha$ in HEK 293T cells. Results are represented by means of $n=3$ independent samples \pm SEM. * $p<0.05$ as measured by one-way ANOVA analysis completed with Dunnett's post-hoc test.

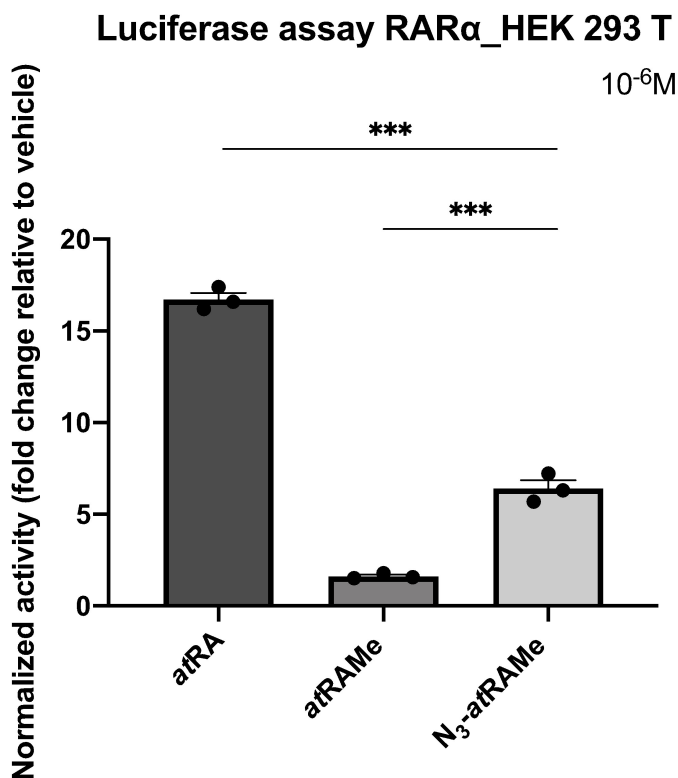


Figure 5. Luciferase assay with 10^{-6} M *atRA* and *atROLAc* as positive controls, compared to *atRAME* and N_3 -*atRAME* for their activation of $RAR\alpha$ in HEK 293T cells. Results are represented by means of $n=3$ independent samples \pm SEM. *** $p<0.001$ as measured by one-way ANOVA analysis complemented with Dunnett's post-hoc test.

we were able to identify the expected click adduct by HPLC-MS analysis of the soluble fraction after plasma extraction using a suitable organic solvent (Figure S12). It is noteworthy that this chemical functionalization is quite fast, since no more free N_3 -*atRAL* could be detected in the medium after 1 hour at 37 °C. This is a highly valuable parameter for this field of application.

Moving to the *in vivo* activity, zebrafish model was used to investigate the functionality of these azido-retinoids. In fact, retinoids are known to induce teratogenic effects during embryonic development.^[50,51] Therefore, zebrafish embryos were exposed to N_3 -*atRAL* or parent retinoid *atRAL* and compared to *atRA* as reference molecule for teratogenicity effects. Zebrafish embryos exposed to increasing concentrations of N_3 -*atRAL* display dose-dependent developmental alterations undistinguishable from that obtained with unmodified parent retinoid *atRAL* (Figure 6). These developmental defects included curved and truncated body axis including tail both proportional to the retinoid's concentrations. Also, the eyes and overall, the head were malformed. Higher concentrations of N_3 -*atRAL* were more severely teratogenic and induced a greater truncation of the embryos with complete loss of the eyes similarly to effects observed for *atRAL*. These findings show that the N_3 -*atRAL* is biologically active and its activity is comparable to the parent retinoid (*atRAL*). For the same concentration and duration of treatment, *atRA*-treated embryos displayed stronger teratogenic malformations leading to severely truncated embryos. These findings correlated with previous findings showing that precursors of RA are less teratogen than RA in zebrafish.^[52]

To visualize these retinoids in zebrafish embryos, click chemistry reactions were conducted on zebrafish embryos preincubated with either N_3 -*atRAL* or the parent retinoid (*atRAL*) (Figure 7A). Confocal imaging shows clear fluorescent signal in N_3 -*atRAL*-treated embryos that is absent in *atRAL*-treated embryos (Figure 7B), suggesting a specific labeling of

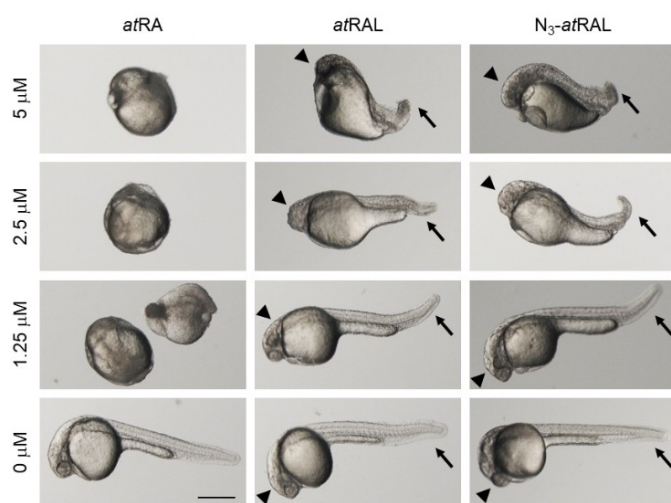


Figure 6. Teratogenic malformations of zebrafish embryos treated with azido-retinoids. While control embryos display normal development, *atRAL* and N_3 -*atRAL* displayed comparable dose-dependent teratogenic malformations including curved spinal cord, truncated tail (arrows), and head malformations (arrowheads). *atRA*, used as positive control, leads to severely truncated embryos. Scale bar = 400 μ m.

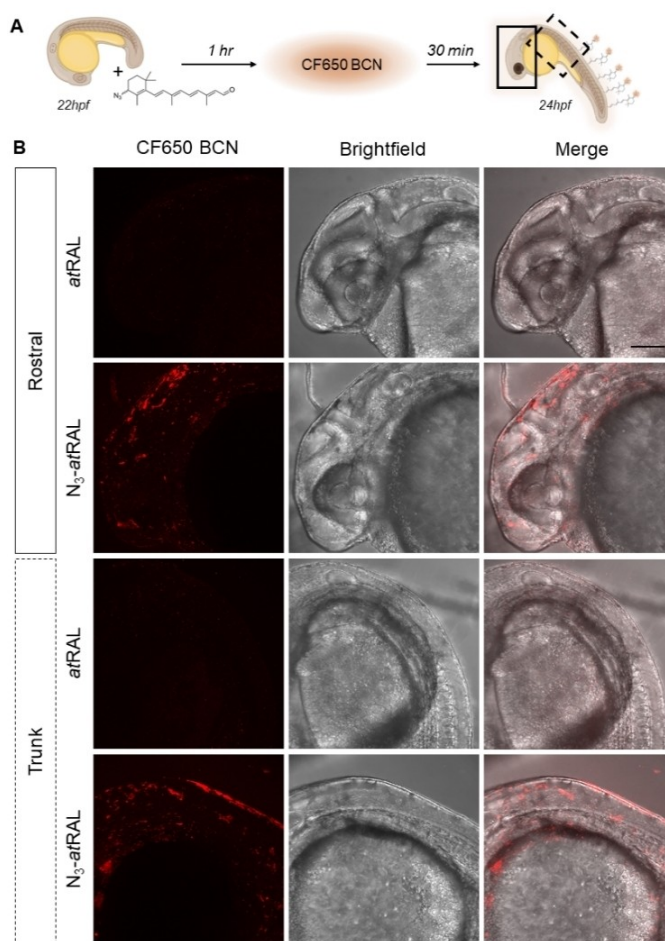


Figure 7. Visualization of azido-retinoids in zebrafish embryos by click chemistry reaction. (A) Schematic illustration of biorthogonal click reaction performed in zebrafish embryos. (B) Illustrative confocal images showing high fluorescence of CF650-BCN dye in zebrafish embryos pretreated with N_3 -*atRAL* but not *atRAL*. Scale bar = 100 μ m.

N_3 -*atRAL* using the biorthogonal BCN dye. Interestingly, the labeling in the hindbrain region and in the trunk correlates with previous findings reporting the expression of *aldh1a2*, which encodes a retinaldehyde dehydrogenase providing the major source of RA in these regions at this specific stage of development.^[53,54] Together, these findings suggest that our biologically active azido-retinoids accumulated in zebrafish embryos can be visualized using biorthogonal click chemistry in complex biological environment such as the developing zebrafish embryo.

In summary, we provide here procedure for rapid and efficient modification of endogenous and synthetic retinoids or their precursors by addition of azide group at C4 position. Our data based on structural biology analyses, functional transactivation tests in cells cultured *in vitro*, and *in vivo* activity testing indicate that azide addition at position C4 does not abolish activity of endogenous and synthetic retinoids. Accordingly, azido-retinoids displayed on average about two-fold higher transactivation as compared to non-modified, parent retinoid molecules. This gain of activity can be explained by

higher binding efficiency caused by reinforced interactions of azido-retinoids with RAR LBD indicated by docking experiments. Thus, our proposed azide insertion seems to be a good strategy to generate retinoid derivatives suitable for click-chemistry based studies of retinoid signaling or click-reaction-controlled pharmacological approaches as previously suggested.^[55]

Conclusions

We reported a new late-stage method for the regioselective insertion of azide group on an allylic position of different retinoids and carotenoids. We performed several experiments to suggest a relevant mechanism in accordance with experiments. We demonstrated the different functionalized retinoid molecular probes retain their ability to efficiently activate genes transcription, despite azide insertion. The present work opens perspectives concerning the preparation of other retinoid molecular probes to be used for studies on vitamin A's metabolism or new strategies of pharmacological applications.

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available in the supplementary material of this article.

Keywords: molecular probes · retinoids · vitamin A · Late stage azidation

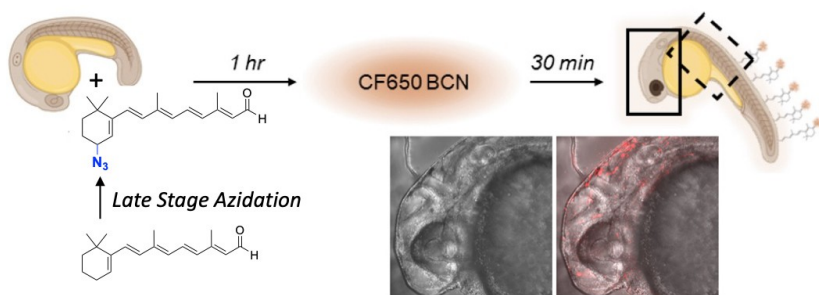
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RESEARCH ARTICLE



A family of close-to-native retinoid probes was synthesized through versatile late-stage azide insertion at the C4 position. These probes retain the ability of the original compound

to activate gene transcription. Their ability to undergo *in vivo* click reactions enabled the highlighting of biological activities in a zebrafish model.

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1 – 9

Retinoids Molecular Probes by Late-stage Azide Insertion – Functional Tools to Decrypt Retinoid Metabolism

