ORGANIC LETTERS

2009
Vol. 11, No. 21
4822−4825

Straightforward Synthesis of the Near-Infrared Fluorescent Voltage-Sensitive Dye RH1691 and Analogues Thereof

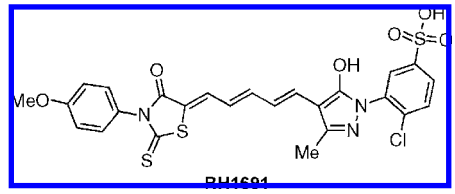
Raphaël Lebeuf, Isabelle Férézou,[†] Jean Rossier,[†] Stellios Arseniyadis,* and Janine Cossy*

Laboratoire de Chimie Organique, ESPCI ParisTech, CNRS, 10 rue Vauquelin, 75231 Paris Cedex 05, France, and Fondation Pierre-Gilles de Gennes pour la Recherche, 29 rue d'Ulm, 75005 Paris, France

stellios.arseniyadis@espci.fr; janine.cossy@espci.fr

Received August 8, 2009

ABSTRACT



A highly straightforward synthesis of the near-infrared voltage-sensitive dye RH1691 is reported featuring two sequential anionic additions of *C*-nucleophilic heterocycles on a cyanine. This convergent approach led to the synthesis of four new probes, which also exhibit fluorescence in the near-infrared region.

One of the most challenging objectives of modern neuroscience is to understand the neuronal bases of behavior. It is therefore crucial to perform functional studies of the cerebral cortex as it is the most integrative structure of the brain involved in many cognitive functions such as processing of sensory information and generation of motor commands. During the past two decades, massive efforts have been made to record single cortical neurons in the intact brain and to investigate how the properties of these neurons and their intricate synaptic connections combine to form networks allowing the processing of information. Recently, the remarkable development of real time cortical imaging[1] with voltage-sensitive dyes[2] has opened new avenues in this field allowing the recording of cortical ensemble activity with a temporal resolution reaching a millisecond and a spatial resolution of a few tens of micrometers.[2b,3−5] This experimental approach is based on the use of dyes that bind to the external surface of excitable membranes without interrupting their normal function and act as transducers that transform changes in membrane potential into optical signals. Various molecules presenting such properties have been developed,[6]

[†] Laboratoire de neurobiologie et diversité cellulaire, ESPCI ParisTech, CNRS, 10 rue Vauquelin, F-75231 Paris Cedex 05, France.

10.1021/ol901846g CCC: $40.75
Published on Web 09/25/2009

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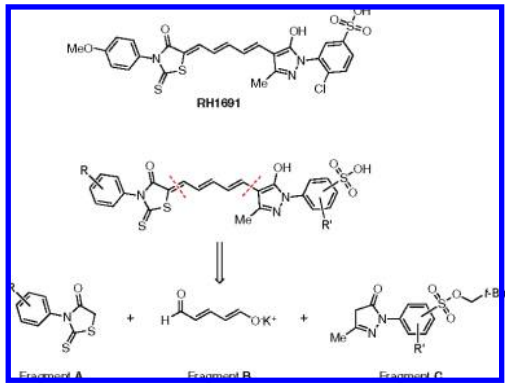
however, most of them still suffer from limitations linked to their intrinsic physicochemical properties. In this context, the development of new high quality voltage probes which allow real time imaging of cortical spatiotemporal dynamics in vivo remains of great interest.

Among all the dyes reported in the literature, we were particularly interested in the "blue" oxonol dye RH1691[7] developed by Grinvald[1h] and co-workers which exhibits an excitation wavelength that has minimal overlap with the absorption of tissues thus conferring minimal pulsation artifacts. Our interest for this dye was initially triggered by the fact that, despite its extensive use, no synthesis had been reported in the literature. In addition, we were particularly motivated in developing an expedient and highly versatile synthesis of this dye, which would offer an easy access to other structurally related near-infrared voltage-sensitive probes that may be more potent and less prone to photodynamic damage or dye bleaching than RH1691. Herein, we report the results of our endeavor.

Our strategy for the synthesis of RH1691 relied on two main disconnections (Scheme 1). Hence, two sequential anionic additions of *C*-nucleophilic heterocycles of type **A** and **C** on a polyene precursor such as glutaconaldehyde[8] (**B**) were envisioned in order to access the entire carbon backbone of RH1691 and analogues thereof.

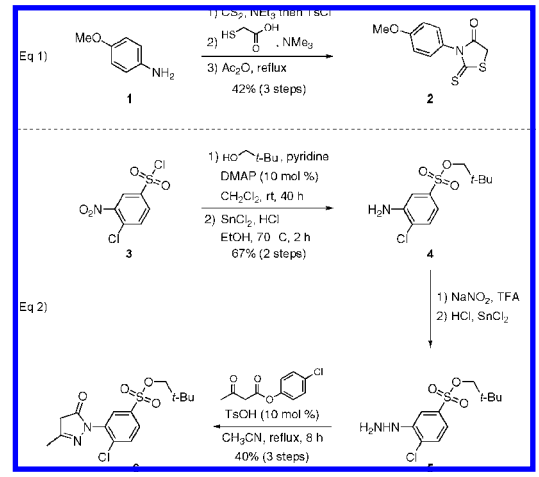
Scheme 1. Retrosynthetic Analysis of RH1691 and Analogues Thereof



The synthesis of **2** was achieved in three steps and 42% yield starting from the corresponding aniline. Hence, 4-methoxyaniline (**1**) was first converted into its corresponding isothiocyanate following the procedure developed by Wong and Dolman.[9] The isothiocyanate was then treated with thioglycolic acid and the resulting dithiocarbamate subsequently cyclized into the corresponding rhodanine by refluxing in acetic anhydride (Scheme 2, eq 1).[10,11]

Compound **6**, on the other hand, was obtained via a key condensation between a β-ketoester and a hydrazine which

Scheme 2. Synthesis of the Two Heterocyclic Coupling Partners



was prepared from commercially available 4-chloro-3-nitrobenzenesulfonyl chloride (**3**) (Scheme 2, eq 2). The choice of the neopentylsulfonate ester protecting group[12] for the sulfonic acid moiety was prompted by its stability toward a variety of reaction conditions[12b] and its ability to be easily cleaved. Hence, **3** was first treated with neopentyl alcohol in the presence of DMAP to afford the corresponding sulfonic ester. The nitro group was then reduced using HCl in combination with $SnCl_2$, thus leading to the corresponding aniline intermediate, which was then converted to the desired hydrazine. The latter was finally condensed with a β-ketoester in the presence of a catalytic amount of TsOH in refluxing acetonitrile to produce compound **6**. The synthesis of **6** was thus achieved in five steps and 27% overall yield starting

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(7) May be purchased from Optical Imaging Ltd. (http://www.opt-imaging.com).

(8) For a review on the use of glutaconaldehyde potassium salt, see: Becher, J. *Synthesis* **1980**, 589–611.

(9) Wong, R.; Dolman, S. J. *J. Org. Chem.* **2007**, *72*, 3969–3971.

(10) Garraway, J. L. *J. Chem. Soc.* **1961**, 3733–3735.

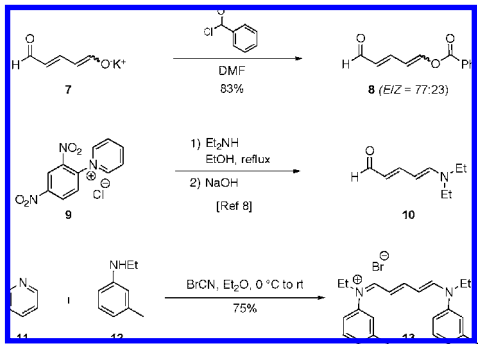
(11) This procedure was preferred to the condensation of bis(carboxymethyl)trithiocarbonate on anilines: Yarovenko, V. N.; Nikitina, A. S.; Zavarzin, I. V.; Krayuskin, M. M.; Kovalenko, L. V. *Synthesis* **2006**, 1246–1248.

(12) Only a few examples of a protecting group have been reported for sulfonic acid moieties: (a) Seeberger, S.; Griffin, R. J.; Hardcastle, I. R.; Golding, B. T. *Org. Biomol. Chem.* **2007**, *5*, 132–138, and references cited therein. (b) Roberts, J. C.; Gao, H.; Gopalsamy, A.; Kongsjahju, A.; Patch, R. *J. Tetrahedron Lett.* **1997**, *38*, 355–358.

from 4-chloro-3-nitrobenzenesulfonyl chloride (**3**) and required only one purification by flash column chromatography at the last stage. It is worth noting that the last step appeared to be much more successful when using a β-ketoester containing an aryloxy leaving group rather than an alkyloxy group.

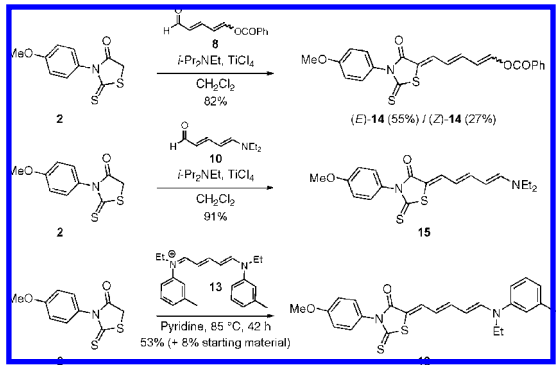
With the two heterocyclic building blocks in hand, we next turned our attention toward the synthesis of the central core of the molecule. As glutaconaldehyde potassium salt **7** is a poor electrophile,[8] the use of its ester derivative **8**,[13] the enamine derivative **10** (Zincke aldehyde),[14] or the related cyanine **13** obtained by ring-opening of pyridine with the corresponding aniline **12**[15] was envisaged (Scheme 3).[16]

Scheme 3. Synthesis of the Central Fragment of RH1691



Both **8** and **10** reacted with rhodanine **2** in the presence of $TiCl_4$ to afford, respectively, the corresponding coupled products **14** and **15** in excellent yields (Scheme 4).[17] A slightly lower yielding but overall more convenient approach involved the use of cyanine **13** which could be condensed with rhodanine **2** to afford the corresponding enamine **16** in 53% yield along with 8% of starting material, which were difficult to separate. Fortunately, the mixture could be used in the next step without further purification.

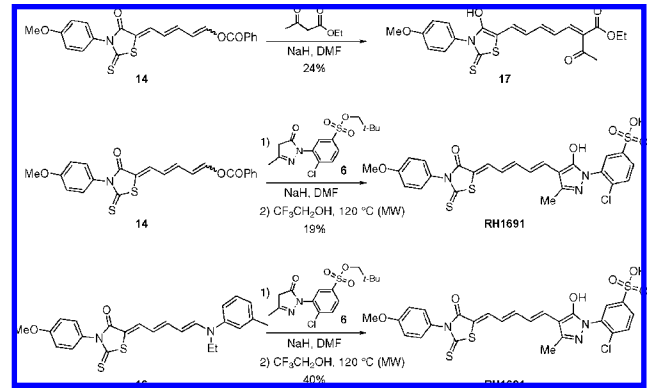
Scheme 4. Synthesis of the Western Fragment of RH1691



While both (*E*)- and (*Z*)-isomers of **14** gave pale yellow solutions, compound **15** displayed a deep-violet coloration [UV absorbance in DCM: $\lambda_{max} = 542$ nm, $\varepsilon = 7.5 \times 10^4$ L·mol^{-1}·cm^{-1}] similar to the one observed for compound **16** [UV absorbance in DCM: $\lambda_{max} = 527$ nm, $\varepsilon = 5.8 \times 10^4$ L·mol^{-1}·cm^{-1}].

The end-game sequence featuring the introduction of the second heterocycle and the final deprotection of the sulfonic acid is illustrated in Scheme 5. As we encountered difficulties converting enol ester **14** into its corresponding aldehyde, we decided to perform a direct nucleophilic displacement of the ester moiety through a 1,8-addition/elimination sequence. To validate this approach, a first try involving ethyl acetoacetate as the nucleophile was performed on substrate **14**. Hence, by treating ethyl acetoacetate with NaH in DMF and adding compound **14** to the resulting enolate, the desired product **17** was isolated in 24% yield. To our delight, when the reaction was performed using **6** as the *C*-nucleophilic heterocycle under otherwise identical conditions, the precursor of RH1691 bearing a neopentylsulfonate ester protecting group (**18**, not shown) was obtained in 19% yield. Interestingly, higher yields were obtained when the reaction was performed on compounds **16** bearing an enamine moiety instead of the enol ester. Indeed, the desired coupled product could now be isolated in a fair 40% yield.

Scheme 5. Condensation of All Three Fragments and Completion of the Synthesis of RH1691



With these results in hand and in order to complete the synthesis of RH1691, the final deprotection of the sulfonic acid had to be performed. Among the various conditions reported in the literature,[12b,18] solvolysis appeared to be the most practical as the free sulfonic acid would be released without generating any undesirable or difficult to remove side products during the process.

We found that heating a solution of the neopentylsulfonate ester in trifluoroethanol at 120 °C under microwave irradiation afforded the desired deprotected product RH1691 quantitatively and without the need of any purification. The spectroscopic data of the synthetic material were identical to those obtained from the purchased compound.

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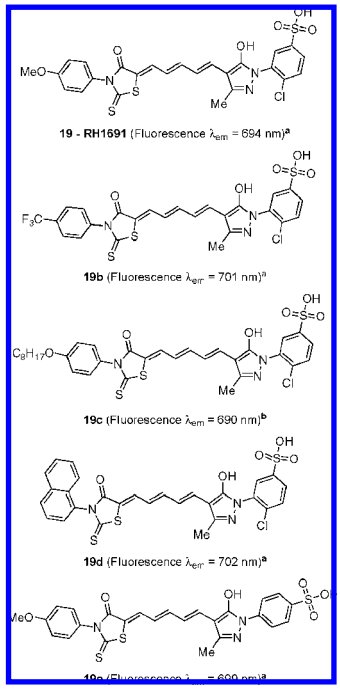

Figure 1. Structure of various RH1691 analogues. (a) Fluorescence was recorded in a phosphate buffer. (b) Fluorescence was recorded in a phosphate buffer/EtOH (3/1) solution.

Having validated the route to RH1691, we embarked in the synthesis of various analogues in order to determine qualitatively a structure−fluorescence relationship (Figure 1). Four new dyes were thus prepared in similar yields, and the fluorescence spectra recorded in a phosphate buffer solution (pH = 7.2). As a general trend, the changes made to both the nature and the position of the substituents on the aryl groups did not have a tremendous impact on the fluorescence emission. However, the disparity in the substitution patterns may be of importance for cell-membrane adsorption or penetration considering the differences in terms of lipophilicity.

In summary, we have completed the synthesis of the near-infrared voltage-sensitive dye RH1691 in seven steps and 11% overall yield via two sequential anionic additions of *C*-nucleophilic heterocycles on a cyanine. In addition to providing the title "blue" oxonol dye RH1691, the described synthetic strategy is scalable and provides a straightforward access to a variety of analogues for real time imaging of cortical spatiotemporal dynamics in vivo. Such studies are currently underway and will be reported in due course.

Acknowledgment. We thank the Fondation Pierre-Gilles de Gennes pour la Recherche for financial support.

Supporting Information Available: Experimental details and characterization data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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(15) (a) König, W. *J. Prakt. Chem.* **1904**, *69*, 105. (b) Reichardt, C.; Stein, J. *Eur. J. Org. Chem.* **1999**, 2899–2908.

(16) Pyrilium perchlorate could also be envisaged; however, for safety reasons, this approach was not investigated. For condensation of pyrilium perchlorate with rhodanine, see: Tolmachev, A. I.; Sribnaya, V. P. *Zh. Obsh. Khim.* **1965**, *35* (2), 316–324 (Eng. Transl.). For its synthesis: Furber, M.; Herbert, J. M.; Taylor, R. J. K. *J. Chem. Soc., Perkin Trans. 1* **1989**, 683–690.

(17) Both stereoisomers of **14** could be separated; however, the exact ratio could not be determined due to the poor solubility of the *E*-isomer compared to the *Z*-isomer.

(18) (a) Roberts, D. D. *J. Org. Chem.* **1999**, *64*, 1341–1343. (b) Koh, H. J.; Lee, H. W.; Lee, I. *J. Chem. Soc., Perkin Trans. 2* **1994**, 253–257.