Xanthene Carboxamides: Indicators for the Assay of n-BuLi and EtMgBr

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Over the last 30 years the use organolithium and organomagnesium compounds have been an essential component for bond forming reactions in organic synthesis. Due to the highly reactive nature of these compounds, the need to readily establish correct titer for a wide variety of organometallic compounds with a simple reagent is highly desirable. Thus, reaction of N-propyl-9-xanthene carboxamide with either n-BuLi or EtMgBr produces a deep orange-red dianion and which can be used as an indicator for the titration of these bases. We have found that the aromatic amide serves well when titrated directly or indirectly when tert-butanol is used as an acid source. Our experiments detail the compound as an indicator for both alkyllithium and alkylmagnesium titrations.

Introduction:

The Gilman double titration method for the assay of alkyllithium reagents remains the gold standard for total organo-metal base determinations.[1] Although the method is precise, it can be difficult to get highly reproducible results for those individuals that are not well practiced in with the technique. This problem has lead to the generation of other methods that commonly eliminate the need for double titrations; often these other techniques eliminate the use of lachrymators and tedious reaction times required for the Gilman assay.

Among these newer methods include: titration using activated halogens,[2] disulfides,[3] titration with colored reversible charge transfer complexes,[4] titration via single deprotonations to give colored anions,[5] titration via double deprotonations to give colored anions,[6] concentration determination via NMR,[7] and titration via cleavage of metal-metal bonds.[8] The majority of these methods work very well within restricted ranges generating the dilemma of which method is optimal for a specific circumstance. This problem suggests the need for a titration method that provides excellent accuracy that spans a very wide range of structural- and metallo- diversity.

We envisioned that real improvements in indicator design could be forthcoming by modifying one of the existing titration methods. Previous work with indicators of very strong bases has demonstrated that color and acidity modulation can be achieved by modifying the electronics of the aromatic rings.[9] However these types of modifications have not been systematically incorporated in the double deprotonation of diphenylacetic acid derivatives. Our strategy was to modify diphenylacetic acid templates into fused structures that would develop an indicator with an enhanced titration profile over existing methods. Our observations with organolithium and organomagnesium reagents suggest that advances in indicator design can be achieved with amide derivatives of xanthene-9-carboxylic acid.

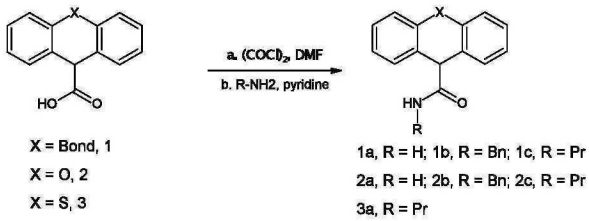

Figure 1: Fluorene, Xanthene and Thioxanene Indicators for Titration

Materials and Methods:

Alklyllithium and Alkylmagnesium reagents were purchased from Aldrich Chemical (P.O. Box 2060 Milwaukee, WI) and used without further purification. *tert*-Butanol was dried over sodium metal and freshly distilled prior to use. Anhydrous tetrahydrofuran was purchased from Aldrich Chemical and used from a Sure/Seal™ Bottle. Water content assayed at <0.002%; inhibitor free. Other reagents were also purchased from Aldrich Chemical and used as delivered. Disposable syringes were purchased from Henke Sass Wolff and used without further drying. Round bottom flasks were dried with the aid of a heat gun and N_2 atmosphere purge. Solution mixing was accomplished by a magnetic stir bar. All column chromatography was carried out using ACROS silica gel 0.20 -0.50 mm, pore diameter – 4 nm. TLC was carried out on Merk Silica Gel Plates with a UV binder. Typical visualization was accomplished by UV, I_2 or $KMnO_4$ staining. ^{1}H NMR was carried out on an Anasazi EFT-60 MHz Spectrometer referenced to TMS. HPLC was carried out using Waters 1525 HPLC with a Waters X-Terra Phenyl column. (pore 5um, length 4.6X150mm). UV Absorbance was detected at at 220 and 245 nm. A linear gradient from 100% solvent A to 100% solvent B was run from time 0 min. to time 16 min. where Solvent A was water:methanol:acetic acid; 9/1/.025 and, Solvent B was water:methanol:acetic acid; 1/9/.025. Melting Points are

uncorrected and were taken on a Fisher-Johns Melting Point Apparatus.

General Method 1: Amide Formation: Used for the formation of compounds 1a to 3a
Typical Example: N-Propyl Xanthne-9-Carboxamide 1c.[10, 11]

A solution of xanthene-9-carboxylic acid (5.00 g, 22.0 mmol) and dichloromethane (50 mL) was treated with oxalyl chloride (3.81g, 30.00 mmol) and dimethyl formamide (2 drops). The reaction mixture was stirred until all gas evolution has stopped (@ 1.5 h). The mixture was stripped on a rotoevaporator and the remaining acid chloride diluted with dichloromethane (100 mL). The slightly yellow solution was cooled to -10 °C and treated with a solution of propylamine (2.56 g, 44.00 mmol), pyridine (3.40 g, 43.0 mmol) in 50 mL of dichloromethane with the aid of a dropping funnel at such a rate to maintain the internal temperature below 5 °C. The mixture was allowed to warm to ambient temperature and stir for 3 h, at which point the mixture was diluted with water, the layers were separated and the dichloromethane fraction washed with solutions of Na_2CO_3 and then $KHSO_4$. The organics were dried over $MgSO_4$ and concentrated to give the crude product as a white solid.

The crude amide was further purified by either silica gel column chromatography with 4:6 ethyl acetate/hexanes as a mobile phase to give the title compound (3.60 g, @ 13.5 mmol 61%); or by crystallization from hot alcohol and water (white needles, 2.5 g, mmmol 43%). TLC (1:3 ethyl acetate/hexane) R_f = 0.3; ^{1}H NMR ($CDCl_3$/ CD_3OD) δ 7.50-6.80 (m, 8 H), 5.90 (s_{br}, 1H), 4.76 (s, 1H), 3.00 (q, 2H, J=6.5 Hz), 1.21 (m, 2H), 0.65 (t, 3H, J=7.2 Hz) ppm. HPLC: Rt: 13.5 min. mp: 192-194 °C

General Methods 2: Procedures for the titration of alkyllithium or alkylmanesium bromide.
Indirect Titration Method:

A 50 mL round bottom flask equipped with a magnetic stir bar, N_2 septum inlet, is charged with 5 mg of **2c**, 10 mL of anhydrous THF, and *tert*-butanol (0.25 g; 3.38 mmol). To the mixture was added organometallic solution dropwise via a graduated syringe. The colorless solution turns orange red near the end point but the color quickly dissipates after a very short time. The endpoint is established by the persistence of the red orange color. All titrations were done in triplicate to verify reliability.

Direct Titration Method:

A 50 mL round bottom flask equipped with a magnetic stir bar, N_2 septum inlet, is charged with **2c** (252 mg, 0.94 mmol) 10 mL of anhydrous THF at -10 °C. To the mixture was added n-BuLi solution dropwise via a graduated syringe. The colorless solution turns orange red near the end point but the color quickly dissipates after a very short time. The color indicates the formation of the dianion. The endpoint is established by the persistence of the red orange color. All titrations were done in triplicate to verify reliability.

General Methods 3: Procedures for the titration of allkyllithium or alkyl magnesium bromide using the Watson- Eastham methods.[4a]

A 50 mL round bottom flask equipped with a magnetic stir bar, N_2 septum inlet, was charged with 3 mg of 1,10-phenanthroline, 10 mL of anhydrous toluene, and *tert*-butanol (0.25 g; 3.38 mmol). To the mixture was added n-BuLi solution dropwise via a graduated syringe. The colorless solution turns rust near the end point but the color quickly dissipates after a very short time. The endpoint is established by the persistence of the color. All titrations were done in triplicate.

A 50 mL round bottom flask equipped with a magnetic stir bar, N_2 septum inlet, was charged with 3 mg of 2,2'-biquinoline, 10 mL of anhydrous toluene, and *tert*-butanol (0.25 g; 3.38 mmol). To the mixture was added ethylmagnesium bromide solution dropwise via a graduated syringe. The colorless solution turns green near the end point but the color quickly dissipates after a very short time. The endpoint is established by the persistence of the color. All titrations were done in triplicate to ensure accuracy.

Results and Discussion:

As part of a project to generate inhibitors of Microsomal Triglyceride Transfer Protein we needed to prepare a variety of amides related to carboxylic acid amides of Xanthene-, Thioxanthene- and Fluorene-9-carboxylic acid.[12,13] The protocol to prepare these inhibitors invoked a dianion alkylation of these aromatic systems. Interestingly, when these aromatic systems were subjected to dianion formation in THF at -20 °C a variety of colors resulted. The dianions of xanthene amides, thioxanthene amides and fluorene amides were orange red, blood red, and pale green respectively. We observed that the solution `remained colorless when one equivalent of base was added, but when a slight excess of the reagent was present the color change was nearly instantaneous. Further addition of base did not result in any noticeable color variances beyond increased intensity. These initial observations suggested that amides of this variety should lead to excellent indicators of strong bases.

With the goal of finding an indicator that titrates both alkyllithium and alkylmagnesium reagents with an endpoint that maintains superior solubility and color profile over diphenylacetic acid compounds we investigated several derivatives of these aromatic systems. The results

of these titrations are summarized in **Table 1** where the color and solubility of all the amides anions are listed. Fluorene-9-carboxamides (**1a-1c**) were either light yellow or faint green. The indicator color intensity is only marginal better than diphenylacetic acid and does not provide advantages over existing methods. However, these fluorene amide compounds demonstrated superior solubility over the dianions of the carboxylic acids.[14] When the xanthene compounds (**2a-2c**) were evaluated a marked improvement with dianion color (orange red) was realized. This outcome was also obtained with the thioxanthene carboxamide (**3a**) where blood red colors were observed. As with the fluorine compounds, these materials maintained increased solubility and endpoint sharpness over the carboxylic acid precursors.

Table 1:Titrations with Aromatic Amides to Determine Quality

Aromatic Amide (#)	Color	Solubility
DPA	Yellow	Poor
F-$CONH_2$ (**1a**)	Green	Good
F-CONHBn (**1b**)	Yellow	Good
F-CONHPr (**1c**)	Green	Good
X-$CONH_2$ (**2a**)	Orange	Good
X-CONHBn (**2b**)	Yellow	Good
X-CONHPr (**2c**)	Orange-Red	Good
TX-CONHPr (**3a**)	Blood Red	Good

DPA=diphenylacetic acid, F=Flourene, X=Xanthene, TX=Thioxanthene, Bn=Benzyl, Pr=Propyl

As a result of the relative ease of synthesis and excellent indicator color and solubility profile we investigated the use of **2c** as an indicator in both direct and indirect titrations. In **Table 2** the results of base concentration determination are shown. Indicator **2c** was compared to both diphenylacetic acid and 1,10-phenanthroline using the Watson-Eastham method to ensure proper controls. In our hands, diphenylacetic acid (DPA) provided titration molarity that was slightly less than that obtained by either indicator **2c** or by the Watson-Eastham method. However, the results were in generally good agreement with each other with determined molarity being 2.3 M (DPA) and 2.4 M, (1,10-phenanthroline and **2c**).

Table 2: Titrations with Aromatic Amides to Determine Molarity

Indicator	Base,	Bu-OH	Vol (mL)	Calcd M
DPA	n-BuLi	----	1.5	2.3
1,10-P	n-BuLi	3.38	1.4	2.4
X-CONHPr (**2c**)	n-BuLi	3.38	1.4	2.4
X-COBHPr (**2c**)	n-BuLi	d.m.	0.4	2.4
2,2'-B	EtMgBr	3.38	1.9	1.8
X-CONHPr (**2c**)	EtMgBr	3.38	1.9	1.8

DPA=diphenylacetic acid, F=Flourene, X=Xanthene, TX=Thioxanthene, Bn=Benzyl, Pr=Propyl, P=1,10-Phenanthroline, 2,2'-B=2,2-Biquinoline, d.m.=direct titration method

To determine if the xanthene amide indicator would also titrate organomagnesium reagents we investigated the use of **2c** with ethylmagnesium bromide as a Grignard base. When the organomagnesium base was added to a solution of *tert*-butanol and **2c** in THF a distinct color change ensued upon the addition of a slight excess base. As with the organolithium reagents the end point color was orange red and there were no detrimental solubility issues associated with the magnesium salts of **2c**. To validate the endpoint accuracy using **2c**, the Grignard solution was also titrated against 2,2'-biquinoline. The results of both experiments established the Grignard molarity at 1.8 M.

In conclusion, N-Propyl Xanthene-9-Caboxamide (**2c**) is an effective indicator for alkylmagnesium halides and alkyllithium reagents. The indicator works well as in both direct and indirect titrations. The results of titrations using N-Propyl Xanthene-9-Caboxamide (**2c**) compare favorably with controls. Due to the fact that the compound is easily prepared and titrates a range of metallo-diversity, we believe that the xanthenes carboxamides are the reagent of choice for these determinations.

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13. The thioxanthene compound was prepared by metalation of thioxanthene with n-BuLi and quenching the anion with carbon dioxide. The resulting acid was then converted into the amide as described in **General Method 1**.
14. The lithium dianions of the acids of xanthene, and thioxanthene were evaluated for solubility and color. There was no advantage over diphenylacetic acid.
15. a) Generous support was provided through NSF Grant 0411383. This grant is in support of NSF HBCU-UP. b) The SC Life Project at Clemson University provided additional resources for undergraduate research. The SC LIFE Project is supported by an award to Clemson University from the Howard Hughes Medical Institute.