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Synthesis and Characterization of Novel Bifunctional Hemithioindigo Chromophores

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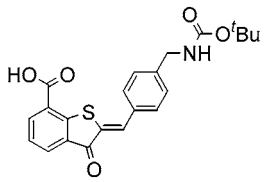
ABSTRACT



General methods for the synthesis of novel bifunctional hemithioindigo (HT) compounds, e.g., ω-amino acid derivatives, are presented. The photochromic properties of the photoswitches have been characterized by UV–vis and ^{1}H NMR spectroscopy.

Photochromic compounds are becoming increasingly popular for a series of biological applications based on the reversible photocontrol of the structure and function of biomolecules.[1] Several approaches to photomodulating biological functions such as biocatalysis,[2,3] ion transport,[4,5] cell adhesion,[6] protein folding,[7] or membrane properties[8] have been employed. Most of these studies involved the chemical modification of nucleotides, peptides, proteins, and lipids using azobenzenes as reversible photoswitchable chromophores.[2−7] In the past, one major strategy for proteins was random-chemical substitution of the chromophore to multiple undefined sites of the biomolecule.[1] Nowadays, rational concepts focus on site-specific incorporation based on the design to photooperate not only steric or ion−dipole interactions but also conformational transitions of structural elements of proteins and peptides as, for example, β-turns and helices.[7,9,10] In this context, novel chromophores with useful optical properties are gaining attention for tailor-made designs. Photochromic hemithioindigos are considered to be an interesting class of chromophore because of the frequent reversibility of the light-induced isomerizations or the thermal stability of the photochromic states among other beneficial properties.[8,11−13] For example, Fyles reported on novel photoswitchable lipids

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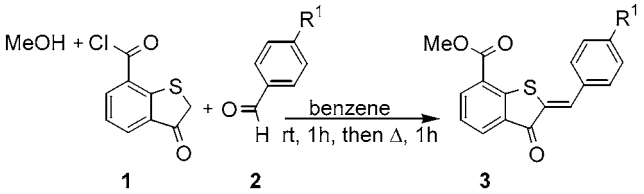
containing a hemithioindigo chromophore as part of the fatty acid for the reversible control of membrane processes.[8] Other hemithioindigo derivatives suitable for incorporation into biomolecules have, to our knowledge, never been explored.

In this report we describe our approach to novel bifunctional hemithioindigo chromophores such as dicarboxylic acid esters and ω-amino acid-derivatives for incorporation into peptides and proteins. We initiated our studies starting from the literature-known thioindoxyl carboxylic acid chloride[14] **1** (Table 1) by examining the use of MeOH and

Table 1. Synthesis of Hemithioindigo-Based Dicarboxylic Acid Esters **3**



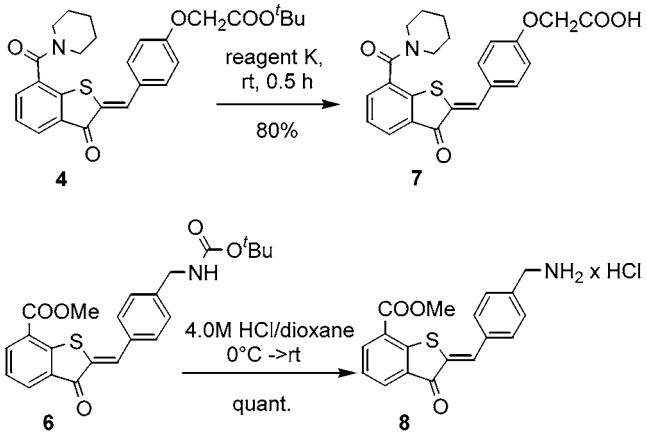
entry	aldehyde	3	R^1	yield[a] (%)
1[b]	**2a**	**3a**	OCH_2CO_2Me	50
2[b]	**2b**	**3b**	CO_2Me	50
3[b,c]	**2c**	**3c**	$OCH_2CO_2{}^tBu$	52

[a] Isolated yields. [b] All reactions were carried out with 0.05−0.15 mL of piperidine as a catalyst. [c] Reaction was performed in the presence of 1.1 equiv of pyridine as an additive.

benzaldehydes **2a**−**c** for the construction of compounds **3a**,**b** as well as the selectively protected dicarboxylic acid ester **3c** via simultaneous esterification in the 7-position and base-catalyzed aldol condensation in the 2-position. Various reaction conditions were examined, and a brief summary of the results obtained is presented in Table 1. The reactions of the thioindoxyl **1** with benzaldehydes **2a**−**c** in the presence of methanol were generally carried out with piperidine as a catalyst.[11] Hemithioindigos **3a** and **3b** were isolated in 50% yield after crystallization from 1:1 toluene/MeOH as yellow and orange powders, respectively, free from impurities and decomposition products. Chromatography on silica gel led to decomposition.

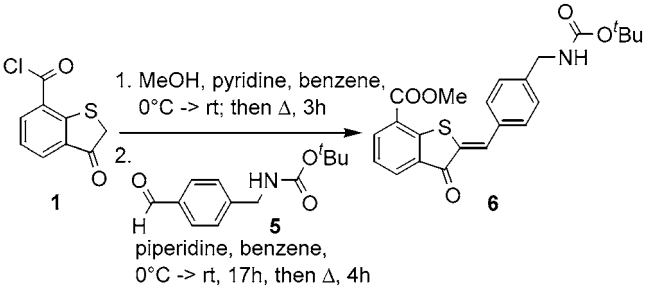
By far, the cleanest reaction yielding **3c** was obtained in the presence of 1.1 equiv of pyridine (52%, recrystallized from ether/pentane). Otherwise, the formation of substantial amounts of byproduct **3a** was found, furnishing an inseparable mixture of **3c** and **3a** in 64% yield in a ratio of 37:63 as determined by ^{1}H NMR. Formation of **3a** can be explained by *tert*-butyl ester cleavage and transesterification in the presence of hydrogen chloride during esterification of the carboxylic acid chloride. With triethylamine as an additive instead of pyridine, decomposition of thioindoxyl **1** took place in the presence of aldehyde **2c** and methanol. Increasing the amount of piperidine (2.3 equiv) in the absence of pyridine afforded amide **4** (Scheme 1) in 54% yield (recrystallized from MeOH).

Scheme 1



We then prepared hemithioindigo ω-amino acid derivative **6** starting from **1**, aldehyde **5**, and methanol by applying exactly the same conditions as for **3c**. But surprisingly the reactions produced inconsistent results regarding yield and purity (entry 1, Table 2). Thus, the reaction conditions were

Table 2. Synthesis of the ω-Amino Acid Derivative **6**



entry	MeOH (equiv)	**1** (equiv)	**5** (equiv)	pyridine (equiv)	conversion of **5** (%)	yield (%)[a]
1	5	1.0	1.1	1.1	61	79
2	5	1.0[b]	1.0	13	81	98
3	7.5	1.5[b]	1.0	19.5	98	98

[a] Yield of isolated crude material **6**. [b] Reaction was carried out in the presence of 4 Å MS.

optimized with a sequential reaction protocol of esterification of thioindoxyl carboxylic acid chloride **1** prior to the

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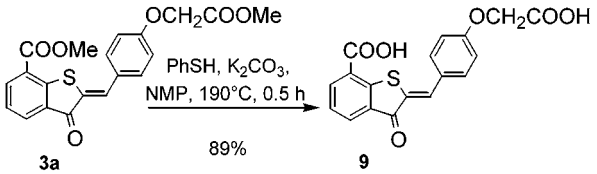
piperidine-catalyzed aldol condensation in degassed solution. Turnover and yield of ω-amino acid derivative **6** were improved up to 98% by increasing the number of equivalents of MeOH and pyridine (entries 2 and 3, Table 2) in the presence of 4 Å molecular sieves. The material contained only tiny amounts of byproduct, presumably due to the oxidation of excess thioindoxyl precursor and esterification intermediate. However, deprotection strategies were investigated with the functionalized hemithioindigos **3a**−**c** and **4** before further optimizations of the reaction protocols.

Deprotection of hemithioindigo *tert*-butyl esters with 1:1 TFA/DCM and 90:2:2 TFA/TIPS/H_2O produced no isolable compounds.[15,16] The decomposition is explained as a consequence of carbocation attack on sulfur and/or hydride attack on the α,β-unsaturated carbonyl moiety. Alternatively, reactions employing reagent K (TFA/water/thioanisole/phenol/ethanedithiole) at room temperature were very promising and gave, for example, compound **7** in 80% yield (Scheme 1). However, for deprotection of the *N*-Boc-protected hemithioindigo compound **6**, treatment with HCl in dioxane proved to be superior, yielding the salt **8** quantitatively.

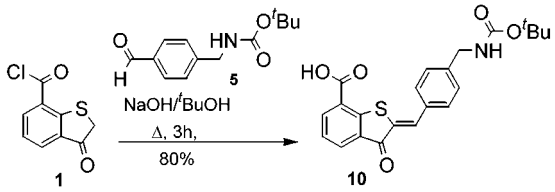
All attempts to selectively deprotect hemithioindigo methyl esters proved to be a challenge. Experiments under basic conditions (LiOH/THF, KO^{t}Bu/Et_2O/H_2O, NaOH/MeOH, $Ba(OH)_2$/MeOH, NaOH/DMF/H_2O) failed.[15,16] Decomposition of the hemithioindigo core unit was observed, presumably via retro-aldol reaction because of the presence of the aldehyde precursors determined by ^{1}H NMR spectroscopy of the crude reaction products. Similarly, exposure of **3a** to Me_3SiCl/NaI in MeCN or NaCN/LiI in DMF at elevated temperatures led to decomposition.[15−17] Fortunately, the application of PhSH and K_2CO_3 in NMP at 190 °C gave product **9** in 89% yield (Scheme 2).[18]

Scheme 2



However, this deprotection procedure requires careful purification of the starting material. Therefore, the direct synthesis of *N*-Boc-protected ω-amino acid **10** was investigated next (Scheme 3). In a final series of experiments, reaction of **1** (1.5 equiv) with benzaldehyde **5** (1 equiv) in 7:3 NaOH(1%)/^{t}BuOH performed very well. Heating at reflux led to complete consumption of the aldehyde within

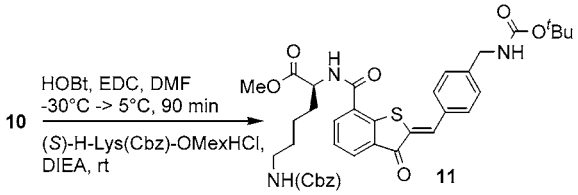
Scheme 3



3 h. Compound **10** was purified by chromatography on Florisil and isolated in 80% yield.

First attempts to apply *N*-Boc-protected ω-amino acid **10** in peptide synthesis proved to be successful. Treatment of **10** with (*S*)-*H*-Lys(Cbz)-OMexHCl in the presence of HOBt, EDC, and DIEA in DMF furnished dipeptide **11** in 88% isolated yield (Scheme 4).[7g]

Scheme 4



After demonstrating the feasibility of the synthesis of bifunctional hemithioindigo compounds, we evaluated their photochromic properties in organic solvents. The UV−vis spectra of **3a**,**b**, **9**, and **10** in DCM or MeOH in the dark adapted state showed a typical maximum at about 438−444 nm, as expected for the (*Z*)-isomers according to previous work (±5 nm).[8,11,13] Irradiation at 406 nm causes the appearance of a broad maximum of the (*E*)-isomers at about 447−461 nm in either DCM or MeOH (Table 3). Presumably, the data collected in Table 3 refer to general observations related to (a) differences in the substitution pattern in the 4′-position at the phenyl residue and (b) differences in the polarity of the solvents as well as (c) in the functional group polarity.[8,11−13]

In all cases, photoisomerization is readily observed at 406 nm (*Z*-to-*E* isomerization) and at 480 nm (*E*-to-*Z* isomerization) showing isosbestic points and reversibility during irradiation for several days. The quantitative analysis of the isomer ratios in the photostationary states (pss) at 406 and 480 nm was determined by ^{1}H NMR spectroscopy, since, e.g., the vinyl protons of both isomers give diagnostic signals in the ^{1}H NMR spectra (Table 3). In the dark adapted state for compound **3a** (CD_2Cl_2) and **10** (CD_3OD), the (*Z*)-isomer is observed exclusively, whereas for compound **9** (CD_3OD), the *Z:E* ratio is 93:7. The thermal *E*-to-*Z* isomerization proceeds rather slowly with a half-life of 138 h for **3a** in CD_2Cl_2 and of 20 and 22 h for **9** and **10**, respectively, in CD_3OD at 25 °C. For the 7-alkoxy carbonyl-substituted hemithioindigos evaluated in this study, more enriched

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Table 3. Photochromic Properties of Hemithioindigo Compounds at Room Temperature

compound	solvent	λ_{max} (nm) Z	λ_{max} (nm) E^d	ϵ ($dm^3\ M^{-1}\ cm^{-1}$) (*Z*)-isomer	isosbestic points	C_E[a,b] (%)	$t_{1/2}$ (h)	C_Z[a,c] (%)
3a	CH_2Cl_2	440	450	13830	376,455	84[e]	138[e]	95[e]
3a	CH_3OH	438	457	7453	379,456			
3b	CH_2Cl_2	438	447	10846	365,454	90[e]		
9	CH_3OH	444	461	8667	481,461	80[f]	20.5[f]	81[f]
10	CH_2Cl_2	438	450	6290	366,449			
10	CH_3OH	442	450	6155	365,457	82[f]	22[f]	81[f]

[a] Ratios were determined by ^1H NMR spectroscopy. [b] Conversion to the (*E*)-isomer determined in the pss (λ = 406 nm). [c] Conversion to the (*Z*)-isomer determined in the pss (λ = 480 nm). [d] Determined in the pss upon illumination with 406 nm. [e] CD_2Cl_2, 25 °C. [f] CD_3OD, 25 °C.

samples in both isomeric states and improved thermal stabilities were determined under illumination in comparison to 4-, 5-, and 6-alkyl-substituted hemithioindigos reported previously.[8,11]

In conclusion, we have synthesized and characterized novel photochromic hemithioindigo-based dicarboxylic acid esters and ω-amino acid derivatives. These chromophores exhibit useful optical properties for the photocontrol of the structure and function of biomolecules. We are currently evaluating the application of **10** in solid-phase peptide synthesis of photoswitchable peptides.

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Supporting Information Available: Full experimental procedures and characterization data for **3a**−**c**, **4**, and **6**−**11**. This material is available free of charge via the Internet at http://pubs.acs.org.

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