Colorimetric Cyanide Chemosensor Based on 1’,3,3’,4-Tetrahydrospiro[chromene-2,2’-indole]

Miglė Dagiliene,[a, b] Vytas Martynaitis,[a] Vilija Krisčiūniene,[a, b] Sonata Krikštolaitytė,[b] and Algirdas Šackus*[a, b]

A new class of chemosensors based on the 1’,3,3’,4-tetrahydrospiro[chromene-2,2’-indole] ring system, which detects cyanide with high specificity, is described. These chemosensors show a distinct color change when treated with cyanide in acetoni-trile solution buffered with sodium phosphate, and this procedure is not affected by the presence of other common anions. The chemisensors exhibit high sensitivity to low concentrations of cyanide, meeting the European Union water quality control criterion of sensitivity below 0.05 mg L⁻¹, and show a very fast response within tens of seconds. The mechanism for detection is rationalized by the nucleophilic substitution of the phenolic oxygen atom at the indoline C-2 atom by the cyanide anion to form a stable indolylnitrite adduct and to generate the colored 4-nitrophenoletate chromophore. These chemosensors can be synthesized by a simple procedure from commercially available starting materials.

Introduction

Cyanide (CN⁻) is a hazardous chemical that leads directly to death in humans, even at low concentrations.[1, 2] Chemical substances containing cyanide occur naturally[3, 4] or can be prepared artificially for use in various areas of industry, including mining,[5] plastics manufacturing,[6] and electroplating.[7] Cassava (Manihot esculenta) plants, which are consumed by millions of people as a source of food,[8, 9] contain cyanogenic glucosides, such as linamarin.[10] Linamarin can be digested by linamarase, a naturally occurring enzyme, or hydrolyzed chemically to liberate highly toxic hydrogen cyanide.[11] Therefore, the presence of cyanide from raw carbohydrates containing cyanogenic glucosides in food products has to be tightly controlled.[2] However, much more concerning are the cyanides used in industries, such as mining, where there is a high risk of contamination of drinking water sources, especially in the process of gold separation.[13] It is well documented that over the last few decades, there have been many cases of transportation accidents and industrial dam failures, where large quantities of cyanide have entered river systems severely impacting aquatic life.[5] In accordance with the the World Health Organization (WHO) recommendations, water containing more than 0.07 mg L⁻¹ (27 × 10⁻⁷ m) cyanide should not be used as a domestic supply,[14] while the European Union (EU) Drinking Water Directive has set the maximum contaminant level for cyanide in drinking water at 0.05 mg L⁻¹ (19 × 10⁻⁷ m).[15] These low limits require extremely sensitive methods for cyanide detection.

In order to control the presence of cyanide in food, feedstock, drinking water, and the environment, numerous studies have focused on the development of methods for its detection, including the use of chemosensors in which a change in color or fluorescence is monitored.[16] Many previous designs for cyanide sensors relied on ditopic binding with crown ether conjugates, hydrogen-bonding interactions, or copper–cyanide affinity.[17, 18] In recent years, there has been increasing interest in the development of chemosensors based on the nucleophilic addition reactions of the cyanide anion to an electrophilic substrate to yield the covalent C–CN bond and the formation of a stable colored species. For example, the treatment of colorless [1,3]oxazine derivatives[19, 20] with cyanide anions yields adducts that absorb at approximately 430 nm, which is in the visual part of the spectrum.[21, 22] 2,8-Dinitroindolo[2,1-b] [1,3]benzoxazine 1 was commercialized by Sigma–Aldrich as a sensitive chemosensor for the detection of cyanide through the formation of a stable colored adduct (2), possessing the 4-nitrophenoletate chromophore (Scheme 1).[23]

It was recently shown that 6-nitro-1’,3’-dihydrospiro[chromene-2,2’-indole] 3, which is well known for its photochromic properties,[24] also behaves as a selective and sensitive cyanide receptor in aqueous media.[25] This compound is converted to the open-ring form upon UV irradiation, and when cyanide anions are present in a solution adduct 4 is produced, resulting in the formation of the colored 4-nitrophenoletate chromophore causing a new absorption band to appear. However, adduct 4...
is not stable and reverts back to its spirocyclic ground state upon visible light irradiation (Scheme 2).

This study is focused on the design and investigation of a new class of highly sensitive, selective and stable chemosensors based on the 1',3',3',4-tetrahydrospiro[chromene-2,2'-indole] ring system.\[26\] Recently, 1',3',3',4-tetrahydrospiro[chromene-2,2'-indoles], which are notable for the single C3–C4 bond in their pyran ring, have been described as ultrafast light-driven switches.\[27\]

**Results and Discussion**

**Synthesis**

The synthesis strategy for 1',3',3',4-tetrahydrospiro[chromene-2,2'-indoles] 8a–h outlined in Scheme 3 is based on the alkylation of Fisher's bases 6a–h with 2-chloromethyl-4-nitrophenol followed by work-up of the intermediate salts (7a–h) with base. The preparation of compounds 8a–cf,g and their characterization was previously described,\[27\] while compounds 8d,e,h are new 1',3',3',4-tetrahydrospiro[chromene-2,2'-indole] derivatives. The preparation of the starting material, 1,2,3,3-tetramethyl-5-nitroindolium iodide 5e, was described elsewhere.\[28\]

1',3',3',4-Tetrahydrospiro[chromene-2,2'-indoles] 8a–h are chiral compounds with a single center of asymmetry. The single-crystal X-ray analysis of compound 8a confirmed that the molecule consists of indole and benzopyran moieties that reside in near-perpendicular planes connected through the chiral spiro C-2' atom (Figure 1).[29]

It was shown previously that 1',3',3',4-tetrahydrospiro[chromene-2,2'-indoles] 8a easily undergoes interconversion of the (R)- and (S)-enantiomers when dissolved in organic solvent due to the thermally induced C(2')–O bond cleavage with formation of the intermediate planar 3H-indolium cation and subsequent ring-closure via the attack of the phenolate negatively charged oxygen atom on either face of the aforementioned planar intermediates. In sodium-phosphate-buffered acetonitrile solution, compound 8a exists in an equilibrated mixture with the open form.\[27\]

The characteristic signal of compounds 8d,e,h in the 1HNMR spectra taken in deuterated chloroform at room temperature was a joint signal of 3,3-methyl groups in the area of 1.24–1.37 ppm, which reflects the coalescence of the separating singlets of the diastereotopic geminal methyl groups due to the inversion at the chiral spiro carbon atom similar to that of known compounds 8a–c,f,g.

**Chemosensing mechanism**

Steady state absorbance spectra of compounds 8a–h, measured for solutions in the acetonitrile/phosphate buffer, revealed absorption in the UV region of the electronic spectra (Table 1). However, when a sodium cyanide solution, buffered with sodium phosphate (pH 7.6), was added to the aforementioned solutions of compounds 8a–h (0.1 mM of 8a–h, 1 mM NaCN in the cell), a new absorption band was observed in the visible area at approximately 420 nm (Table 1, Figure 2).

The appearance of this band in the visible region can be rationalized by spirochromene ring opening and formation of 4-nitrophenolate chromophores 9a–h due to the nucleophilic substitution of the phenolic oxygen by a cyanide group. In this
In this case, the limiting step of the reaction is the C–O covalent bond cleavage and the formation of intermediates B, which are in an equilibrated mixture with the starting 1',3',3'-tetrahydrospiro[chromene-2,2'-indoles] (8a–h). However, intermediates A are quickly consumed when the nucleophilic addition of the cyanide anion to the C-2 carbon occurs to form stable nitriles 9a–h. In such cases, the ring-opening reaction of 8a–h becomes irreversible (Scheme 4).

When compound 8a was treated with sodium cyanide in tetrahydrofuran (THF) containing a small amount of water, the reaction afforded indole-2-carbonitrile 10 (Scheme 5), the structure of which was confirmed by spectroscopic methods and elemental analysis. The IR spectrum of 10 contains an absorption band at 2222 cm⁻¹, characteristic for nitriles. The heteronuclear multiple bond coherence (HMBC) spectrum revealed three bonds range coupling between the methylidene protons at 2.15 and 2.30 ppm and the nitrile carbon at 118.49 ppm. These protons also interact with the quaternary carbon at 76.75 ppm, separated by two bonds, and with the quaternary carbons at 46.76 and 128.16 ppm, separated by three bonds. The full assignments presented in Table 1.

### Table 1. UV-vis spectral data for compounds 8a–h in CH₃CN/phosphate buffer.

| Compd | λ max of 8 [nm] | ε x 10³ [dm³ mol⁻¹ cm⁻¹] | λ max of 9 [nm] | ε x 10³ [dm³ mol⁻¹ cm⁻¹] |
|-------|----------------|---------------------------|----------------|---------------------------|
| 8a    | 205            | 51.4                      | 250            | 17.2                      |
|       | 243            | 16.3                      | 282            | 7.7                       |
|       | 326            | 14.6                      | 422            | 29.0                      |
| 8b    | 205            | 42.3                      | 253            | 12.2                      |
|       | 246            | 14.4                      | 286            | 9.1                       |
|       | 326            | 12.5                      | 420            | 20.7                      |
| 8c    | 206            | 46.3                      | 255            | 41.9                      |
|       | 245            | 15.7                      | 300            | 23.3                      |
|       | 313            | 13.6                      | 422            | 24.8                      |
| 8d    | 210            | 37.2                      | 253            | 10.4                      |
|       | 246            | 10.7                      | 293            | 4.2                       |
|       | 326            | 11.2                      | 422            | 21.8                      |
| 8e    | 229            | 11.8                      | 254            | 13.8                      |
|       | 321            | 12.7                      | 422            | 30.0                      |
|       | 375            | 19.0                      |                |                           |
| 8f    | 230            | 14.1                      | 257            | 14.5                      |
|       | 330            | 14.6                      | 424            | 32.3                      |
|       | 380            | 22.1                      |                |                           |
| 8g    | 205            | 46.1                      | 254            | 42.3                      |
|       | 244            | 16.7                      | 298            | 23.4                      |
|       | 313            | 12.4                      | 419            | 23.4                      |
| 8h    | 205            | 54.3                      | 252            | 31.3                      |
|       | 242            | 17.9                      | 298            | 17.2                      |
|       | 312            | 12.9                      | 418            | 17.6                      |

Figure 2. Absorption (A) spectra of 8a (0.1 mm, 298 K) in a mixture of CH₃CN/phosphate buffer (Na₂HPO₄/NaH₂PO₄, 7.5 mm, pH 7.6) (19:1, v/v) without (spectrum A) and with (spectrum B) NaCN (10 equiv).

Figure 3. ¹H (blue) and ¹³C (red) NMR chemical shifts [ppm] for 10 in [D₆]DMSO.

Scheme 4. Formation of 4-nitrophenolate chromophore 9.

Scheme 5. Formation of 1H-indole-2-carbonitrile 10. Reagents and conditions: a) THF, NaCN, H₂O, rt, 1 h.
based on the combined application of standard NMR techniques such as COSY, NOESY, ROESY, APT, DEPT, HSQC and HMBC spectra.

Response time

For various types of chemosensors, the response time is very important for the practical detection of analytes. In our case, we observed that the response time depends on the structure of the compound. After the addition of sodium cyanide to the solutions of compounds 8a–d, a strong new absorption band at 422 nm appeared within 1–3 min (Figure 4), while for compounds 8e–f, this process took up to 1.5 h (Figure 5). We conclude that the presence of a nitro group at C-5 of the indole nucleus stabilizes the closed form of the molecule and slows down the formation of the final adduct 9e–f. Both electronic and steric effects can be considered for explaining the influence of the allyl and benzyl groups at the indole nitrogen atom on the formation of adducts 9g–h after the addition of sodium cyanide to a solution of compounds 8g–h, respectively. The response time of the reference compound, 2,8-dinitroindole[2,1-b][1,3]benzoxazine 1, was evaluated in analogous conditions, and the formation of colored adduct 2 proceeds more slowly in comparison with compounds 8a–d. The full process takes approximately 20 min (Figure 5), similar to that of compounds 8g–h.

Selectivity and sensitivity

To test the selectivity of chemosensor 8a, parallel investigations were carried out with a series of other anions (F−, Cl−, Br−, I−, CH3COO−, C6H5CO2−, HCO3−, HSO3−, HSO4−, NO2−, NO3−, Br−, I−, SCN−, SO2−, SO3−, S2O3−). The addition of excess amounts of these anions did not result in significant absorbance spectral changes during UV/vis titration (Figure 6), indicating that this potential chemosensor demonstrates excellent selectivity for cyanide over other common anions. The high selectivity of chemosensing can be explained by the fact that only the addition of a cyanide anion on the indoline nucleus forms a strong enough covalent bond with the indole C-2 atom to afford stable nitrile 9, while the addition of the other aforementioned anions is a reversible process.

To evaluate sensitivity, the calibration curve of cyanide concentration versus absorption at 422 nm for 8a was plotted (Figure 7), showing that this chemosensor is sensitive to relatively low concentrations of CN− and meets the European Union Drinking Water Directive criterion for water quality, with sensitivity below 0.05 mg L−1 (19 × 10−7 M).

Conclusion

The derivatives of 1,3,3’,4-tetrahydrospiro[chromene-2,2’-indole], synthesized by a simple procedure from commercially available starting materials, undergo transformations to the
Reagents and solvents were purchased from Sigma–Aldrich and used without further purification. Water was doubly distilled. The anionic solutions for selectivity testing were prepared from sodium salts of various anions.

1,2,3,3,7-Pentamethyl-3H-indolium iodide (5d): 2,3,3,7-Tetramethyl-3H-indole (0.865 g, 5 mmol) was mixed with iodomethane (1.42 g, 10 mmol), and the mixture was heated at reflux for 24 h. The resultant crystalline material was isolated by filtration and recrystallized from EtOH to afford 5d as a brownish crystalline solid (1.12 g, 71%); m.p. 248–250 °C; 1H NMR (300 MHz, TFA-d): δ = 1.65 (s, 6H, 2×3-CH₃), 2.86 (s, 6H, 2-CH₂-7-CH₃), 4.34 (s, 3H, NCH₃), 7.41–7.59 ppm (m, 3H, Ar-H); 13C NMR (75 MHz, TFA-d): δ = 15.6, 20.8, 24.2 (2×CH₃), 40.8, 56.3, 123.3, 129.7, 132.8, 135.9, 142.4, 144.8, 198.1 ppm; IR (KBr): 3024, 2966, 1625 cm⁻¹ (CdbondN⁺); MS (ESI): m/z (%): 188 [M–I]⁺ (100); Anal. calc for C₁₃H₁₈N⁺: C 49.54, H 5.76, N 4.44; found: C 49.61, H 5.37, N 4.24.

Crude 6d was dissolved in CH₃CN (3 mL), and 2-chloromethyl-4-nitrophenol (0.938 g, 5 mmol) was added to the solution. The mixture was stirred at rt for 6 h. The resultant crystalline material was isolated by filtration, washed with cold CH₃CN (1 mL), and dried in vacuo to afford 7d as an off-white crystalline solid (0.907 g, 48%); m.p. 200–203 °C; 1H NMR (300 MHz, D₂O): δ = 1.58 (s, 6H, 2×3-CH₃), 2.78 (s, 3H, 7-CH₃), 2.95–3.01 (m, 2H, CH₂), 3.32–3.38 (m, 2H, CH₃), 4.31 (s, 3H, NCH₃), 7.31 (d, J = 8.9 Hz, 1H, Ar-H), 7.39 (d, J = 7.5 Hz, 1H, Ar-H), 7.5 (t, J = 7.5 Hz, 1H, Ar-H), 7.67 (d, J = 7.5 Hz, 1H, Ar-H), 8.07 (dd, J = 8.9, 2.5 Hz, 1H, Ar-H), 8.35 (d, J = 2.5 Hz, 1H, Ar-H), 12.1 ppm (s, 1H, OH); 13C NMR (75 MHz, D₂O): δ = 19.1, 21.5 (2×CH₃), 26.2, 38.8, 39.0, 53.5, 115.3, 121.1, 124.6, 126.6, 127.2, 129.3, 132.5, 139.2, 140.3, 142.8, 162.3, 192.5 ppm; IR (KBr): 3624 (OH), 3050, 2923, 1520 (NO₂); Anal. calc for C₁₄H₁₃NO₂C₁₂H₂₃N: C 64.08, H 6.18, N 7.47; found: C 64.45, H 6.25, N 7.21.

1,3,3,7-Tetramethyl-6-nitro-1,3,3,7-tetrahydrospiro[cromene-2,2-indole] (8d): A solution of 7d (1.124 g, 3 mmol) in EtOH (5 mL) was diluted with water (15 mL). 10% aq NH₄ solution (~ 0.5 mL) was added dropwise to the solution while stirring until the mixture became turbid. The separated product was extracted with Et₂O (3×20 mL), and the combined extracts were dried over anhydrous Na₂SO₄, filtered, and concentrated in vacuo. The solid residue was recrystallized from CH₃CN to give 8d as a yellowish crystalline solid (0.518 g, 51%); m.p. 206–209 °C (CH₃CN); 1H NMR (300 MHz, CCl₄): δ = 1.24 (s, 6H, 2×3-CH₃), 2.31–2.36 (m, 2H, CH₂), 2.50 (s, 3H, 7-CH₃), 3.05–3.10 (m, 5H, CH₂, NCH₃), 6.76–6.82 (m, 2H, 5–H, 8–H), 6.91–6.96 (m, 2H, 4‘–H, 6‘–H), 7.99 (dd, J = 9.0, 2.7 Hz, 1H, 7–H), 8.04 ppm (d, J = 2.7 Hz, 1H, 5–H); 13C NMR (75 MHz, CCl₄): δ = 20.1, 23.4, 24.0, 24.1, 25.6, 32.0, 48.8, 107.4, 116.7, 119.1, 119.2, 119.8, 121.5, 124.1, 125.1, 131.7, 137.6, 140.5, 146.5, 161.8 ppm; IR (KBr): 3084, 2975, 1513 (NO₂, asymm), 1330 cm⁻¹ (NO₂, symm); HRMS (ESI): m/z (%): 339 [M+H⁺] (100); Anal. calc for C₁₇H₁₄N₂O₂: C 70.99, H 6.55, N 8.28; found: C 70.76, H 6.61, N 8.54.
1',3',3'-Trimethyl-5,6-dinitro-1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] (8e): A stirred solution of iodide 5e (1.69 g, 5 mmol) in distilled water (50 mL) was treated with NaCN (1.012 g, 10 mmol) at rt. The mixture became turbid immediately and was extracted with EtO (3 x 20 mL). The combined organic phase was dried over anhydrous NaSO₄, filtered, and concentrated in vacuo to afford crude intermediate enamine 6e as a brownish oil.

Crude 6e was dissolved in CH₂CN (3 mL), 2-chloromethyl-4-nitrophenol (0.938 g, 5 mmol) was added, and the mixture was stirred for 6 h at rt. Then, the reaction mixture was poured into water (50 mL), and 10%aq NaOH solution (~0.75 mL) was added dropwise while stirring until the mixture became turbid. The separated product was extracted with EtO (3 x 20 mL), and the combined extracts were dried over anhydrous NaSO₄, filtered, and concentrated in vacuo. The residue was subjected to flash chromatography on silica gel (hexane/acetic acid, 4:1 v/v), and the obtained solid was re-crystallized from acetonitrile to give 8e as a yellowish crystalline solid (0.739 g, 40%): Rf = 0.17 (hexane/acetonitrile, 4:1 v/v). m.p. 232–234°C (CH₂CN); 1H NMR (300 MHz, CDCl₃): δ = 1.28 (s, 6H, 2 x 3'-CH₂), 2.29–2.45 (m, 2H, CH₂), 2.96 (s, 3H, NCH₃), 3.03–3.17 (m, 2H, CH₂), 6.65 (d, J = 8.7 Hz, 1H, 7’-H), 6.79 (d, J = 9.0 Hz, 1H, 8’-H), 7.91 (d, J = 2.4 Hz, 1H, 4’-H), 7.99 (dd, J = 9.0, 2.7 Hz, 1H, 7-H), 8.05 (d, J = 2.7 Hz, 1H, 5’-H), 8.17 ppm (dd, J = 8.7, 2.4 Hz, 1H, 6’-H); 13C NMR (75 MHz, CDCl₃): δ = 21.9, 23.3, 24.1, 25.7, 26.8, 49.3, 104.1, 106.0, 117.0, 118.2, 121.3, 125.4, 125.6, 134.0, 134.0, 140.8, 141.3, 154.0, 161.0 ppm; IR (KBr): 3068, 2967, 2929, 1612, 1516 (NO₂); found: C 68.56, H 6.07, N 11.60.

1'-Benzyl-3,3'-dimethyl-6-nitro-1',3,3',4-tetrahydrospiro[chromene-2,2'-indole] (8h): A solution of 2,3,3-trimethyl-3H-indole (1.59 g, 10 mmol) in CH₂CN (10 mL) was treated with benzyl iodide (2.39 g, 11 mmol), and the mixture was heated at reflux for 24 h. After cooling to rt, the solvent was removed in vacuo, and the reaction mixture was kept under high vacuum for 20 min to give crude 5h as a brown amorphous solid, which was used for further reaction without purification.

A stirred solution of crude 5h (1.955 g, 5 mmol) in CH₂Cl₂ (15 mL) was treated with benzyl iodide (2.39 g, 11 mmol), and the mixture was heated at reflux for 24 h. After cooling to rt, the solvent was removed in vacuo, and the reaction mixture was kept under high vacuum for 20 min to give crude 6h as a brownish oil.

Crude 6h was dissolved in CH₂CN (3 mL), 2-chloromethyl-4-nitrophenol (0.938 g, 5 mmol) was added, and the mixture was stirred for 6 h at rt. Then, the reaction mixture was poured into water (50 mL), and 10%aq NaOH solution (~0.75 mL) was added dropwise while stirring until the mixture became turbid. The separated product was extracted with EtO (3 x 20 mL), and the combined extracts were washed with water (3 x 30 mL), dried over anhydrous NaSO₄, filtered, and concentrated in vacuo to afford crude intermediate enamine 6h as a brownish oil.

Analytical procedures

For the measurement of UV/vis absorption, compounds 8a–h were dissolved in a mixture of CH₂CN/phosphate buffer (Na₂HPO₄, NaH₂PO₄, 7.5 mM, pH 7.6) (19:1, v/v, 298 K). Each solution (0.1 mM) was transferred to a spectrophotometer quartz cell (0.5 cm light path length) and 0.005 mL 72 mM NaCN stock solution was added. This volume was negligible compared with the initial volume of the solution in the cell (1.8 mL). The mixtures were shaken, and the absorption was measured from 200 to 600 nm against a blank of CH₂CN/phosphate buffer (19:1, v/v, 298 K).

To construct a calibration curve of cyanide concentration versus the most sensitive absorption at 422 nm for 8a, different cyanide solutions (25, 50, 75, 125, 250, 500 µL of 0.72 mM and 25, 125, 250, 375 µL of 3.6 mM) were added to 100 mL of 0.1 mM solution 8a in CH₂CN/phosphate buffer. A 72 mM NaCN stock solution was prepared from NaCN and diluted to 36 mM, 3.6 mM and 0.72 mM. All of the added volumes of cyanide were negligible, with 0.875 mL as the highest volume, compared with the initial volume of the 8a solution.

**Acknowledgements**

This research was funded by a grant (no. MIP-022/2013) from the Research Council of Lithuania.

**Keywords**

1',3',3',4-tetrahydrospiro[chromene-2,2'-indole] · 2-chloromethyl-4-nitrophenols · 2-methylidene-2,3-dihydro-1H-indoles · chemosensors · cyanide · water quality

[1] Cyanide in Water and Soil: Chemistry, Risk, and Management, (Eds.: D. A. Dzombak, R. S. Ghosh, G. M. Wong-Chong), CRC Press, Boca Raton, 2006.

[2] R. Gracia, G. Shepherd, Pharmacotherapy 2004, 24, 1358–1365.
