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Charge-Compensated Metallacarborane Building Blocks for Conjugation with Peptides

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The cobalt bis(dicarbollide) complex [*commo*-3,3′-Co(1,2-$C_2B_9H_{11})_2$]$^-$ has captured much attention in biochemical and medical contexts, in particular for the treatment of tumors by boron neutron capture therapy (BNCT). Derivatives of cobalt bis(dicarbollide) are commonly prepared through ring-opening reactions of cyclic oxonium ions, so the corresponding products are usually charged. Furthermore, attempts to incorporate cobalt bis(dicarbollide) into peptides are rare, despite obvious potential advantages. Here the synthesis of an imidazolium-based charge-compensated cobalt bis(dicarbollide) building block, which allows additional modifications with moieties of biochemical relevance, such as monosaccharides, is reported. Furthermore, conjugates of these building blocks with the Y_1-receptor-selective derivative of neuropeptide Y ([F^7,P^{34}]-NPY) retained excellent response to hY_1 receptors found to be overexpressed in breast tumors and metastases.

Introduction

The cobalt bis(dicarbollide) anion [*commo*-3,3′-Co(1,2-$C_2B_9H_{11})_2$]$^-$ (**1**, Scheme 2, below) has captured much interest since its discovery by Hawthorne et al.[1] in 1965. In this highly stable anionic complex, the cobalt(III) cation has a d^6 low-spin configuration and is coordinated by two η^5-bonding dicarbollide ligands (1,2-$C_2B_9H_{11})^{2-}$ in a sandwich-type arrangement. At present, its chemistry is one of the most thoroughly elaborated in metallaborane chemistry, and numerous potential applications have been reported.[2] In the past decade, **1** has been intensely studied in biochemical and medical contexts. Derivatives of **1** were shown to be powerful inhibitors of HIV-1 proteases,[3] and modified DNA nucleotides,[4] amino acids,[5] and porphyrins[6] containing the parent moiety **1** have been prepared. Many of them are promising candidates as boron-delivering agents for boron neutron capture therapy (BNCT).

BNCT is based on the idea of delivering boron compounds to tumor tissue, which is subsequently irradiated by nonhazardous, thermal neutrons. These engage in a nuclear reaction with the $^{10}_5$B isotope, which has a natural abundance of about 19.9% and a remarkable capability to capture thermal neutrons, with a cross section of about 3800 barn.[7]

$$^{10}_5B + {}^1_0n \rightarrow {}^{11}_5B^* \rightarrow {}^7_3Li + {}^4_2He$$

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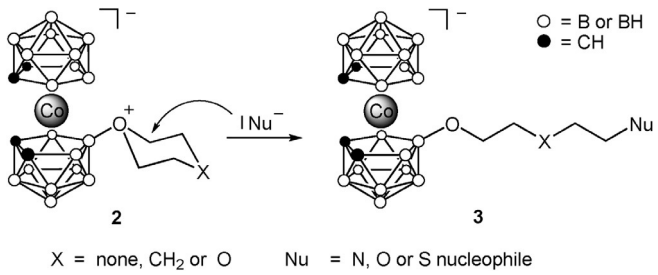
$^7_3$Li and $^4_2$He are particles of high kinetic energy, which cause lethal damage to cells. Because the mean free path of the particles is limited to approximately the diameter of a tumor cell (<10 μm), BNCT is a highly selective method when measures are taken to produce the cytotoxic particles—$^7_3$Li and $^4_2$He—in tumor cells only.[8] Achieving the selective accumulation of boron compounds in tumor cells has proven to be one of the major problems in BNCT, but promising approaches are now well documented. Thus, we found derivatives of neuropeptide Y (NPY)[9] to be selective carrier systems and demonstrated their excellent capability to deliver boron to breast tumor cells. In quantitative studies, the content of boron in the cancer cells was found to exceed the significant amount of 10^9 boron atoms per cell.[10] Besides NPY, several other peptides have been proposed as tumor-targeting carriers: examples include somatostatin, epidermal growth factor, neurotensin, substance P, gastrin-releasing peptide, insulin-like growth factor,[11] α-melanocyte-stimulating hormone, cholecystokinin, vasoactive intestinal peptide, and bombesin.[12]

In view of this large number of peptides, it appears surprising that the cobalt bis(dicarbollide) anion **1** has never been incorporated into any tumor-selective peptide despite obvious possible advantages.[13] The high stability relative to polyhedral boron compounds, the strong lipophilicity (giving rise to hydrophobic peptide–receptor interactions), the hydridic shell (which facilitates unusual molecular interactions unknown in traditional organic scaffolds), and the large number of boron atoms make anion **1** an interesting moiety, in particular for applications in BNCT.[2a,b] The biologically active derivatives of cobalt bis(dicarbollide) that have previously been studied[3–6,13] have exclusively been obtained by ring-opening reactions of cyclic oxonium derivatives **2** (Scheme 1).

This approach has been preferred, due to the high yields and the variety of moieties that can readily be introduced



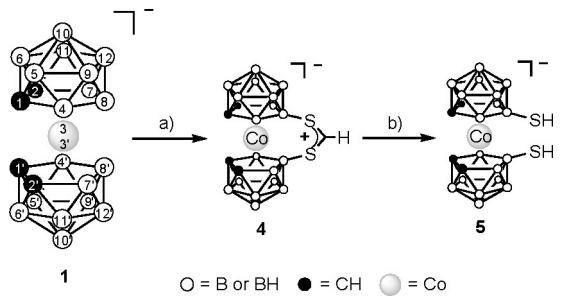
Scheme 1. Common route to biologically active compounds incorporating the cobalt bis(dicarbollide) system **1**. Cyclic oxonium derivatives **2** are opened by nucleophiles.

through the attacking nucleophiles.[14] However, the ring-opened products **3** are negatively charged and require countercations, and this can give rise to perturbation in boron-delivery systems. Ionic compounds cause higher osmotic pressures than neutral compounds in cells (colligative property) and can have a negative impact on the mechanism of agent transport into tumor cells due to ionic interactions. Additionally, the solubilities of the derivatives **3** strongly depend on the cations. Exchange of the cations with ions originating from the biological matrix can result in dramatic decreases in solubility.[14] To avoid these side effects, we designed a new charge-compensated (zwitterionic) key intermediate of **1**, which allows further modifications with moieties of biochemical relevance, such as monosaccharides. The resulting building blocks were successfully coupled to the tumor-selective peptide [F^7,P^{34}]-NPY[10] with retention of excellent Y_1 receptor activity.

Results and Discussion

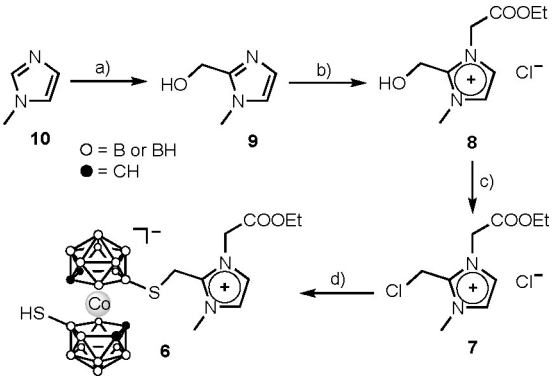
Design and synthesis of the key compound 6

For the design of the new building blocks, our interest was captured by **4**, which has been described by Hawthorne and co-workers (Scheme 2).[15,16] Compound **4** is formed in a remarkable reaction between **1** and carbon disulfide, promoted by aluminum chloride. The C–S bonds of the dithioformate bridge in **4** are readily hydrolyzed under acidic, oxygen-free conditions to give **5**. We saw **5** as a potentially valuable starting material, because both mercapto groups could act as powerful nucleophiles, but surprisingly, no further chemistry of **4** or **5** had been reported.



Scheme 2. a) CS_2, $AlCl_3$, HCl (anhydrous, gaseous), 68%; b) EtOH, HCl (aqueous, dilute, catalytic), quantitative.[15,16] The numbering scheme for the cage positions in anion **1** is given on the left.

To compensate for the negative charge (and to avoid cations), we envisaged combining **1** with a stable imidazolium moiety to form a neutral zwitterion such as **6** (Scheme 3).



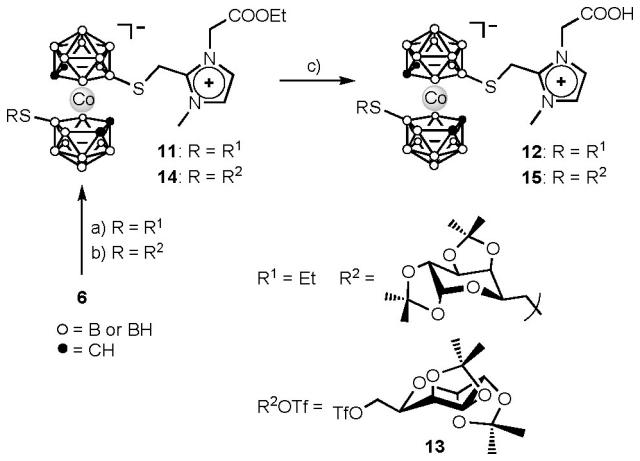
Scheme 3. Synthesis of the key compound **6**. a) Paraformaldehyde, MeCN, sealed tube, 120 °C, 12 h, 69%; b) $ClCH_2COOEt$, EtOH, 60 °C, 12 h, 80%; c) $SOCl_2$, MeCN, reflux, 1 h, 75%; d) **4**, HCl (aqueous, concentrated, catalytic), EtOH, 70 °C, 24 h, 90%.

The required imidazolium salt **7** was obtained through a three-step synthesis. *N*-Methylimidazole (**10**) was hydroxymethylated by a modified procedure.[17] The resulting compound **9** was treated with ethyl chloroacetate with formation of the imidazolium salt **8**. Chlorination of **8** with thionyl chloride gave **7**, containing a reactive chloromethyl moiety.

The reaction between one equivalent of **7** and **5** gave only low yields of **6** (≈30%), although various combinations of bases and solvents were applied. We assume that disubstitution of **7** at both mercapto groups led to side products but did not carry out further studies. A much higher yield of the desired **6** (90%) was achieved when **4** was treated with **7** in the presence of catalytic amounts of acid. Under these conditions, the C–S bonds of the dithioformate bridge are cleaved in situ, and monosubstitution with **7** proceeds selectively to give **6**. The $^{11}B\{^1H\}$ NMR spectrum of **6** shows the typical pattern of two chemically inequivalent dicarbollide ligands. In the solid state, **6** is stable towards air and moisture. However, in solution or under alkaline conditions, **6** is significantly oxidized and dimerizes with formation of a disulfide bridge, indicated by a color change from red to dark purple and by the appearance of signals at −28 ppm in the $^{11}B\{^1H\}$ NMR spectrum.[16]

The pathway towards new building blocks

Compound **6** is the key compound for new building blocks. The mercapto group in **6** can act as a nucleophile and can be used for the introduction of additional moieties as in **11** and **14** (Scheme 4). If no specific modification is required, the mercapto group in **6** should be protected to avoid undesired side reactions such as disulfide formation. Furthermore, the ethyl ester groups in **11** and **14** can be cleaved with alkali metal hydroxides, and the resulting carboxylate groups in **12** and **15**



Scheme 4. a) R^1Br, K_2CO_3, MeCN, RT, 12 h, 90%; b) R^2OTf (**13**), K_2CO_3, MeCN, RT, 12 h, 85%; c) NaOH, MeOH, 30 min, 60 °C, then HCl (aqueous, dilute), CH_2Cl_2, quantitative.

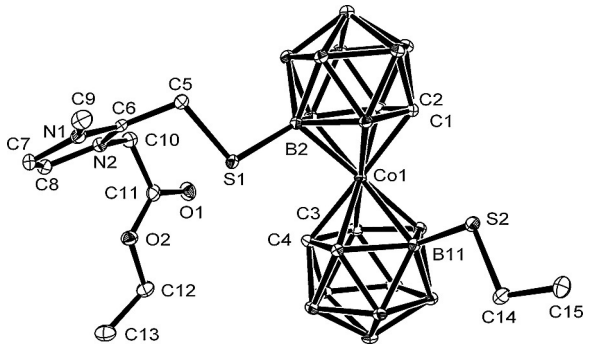


Figure 1. Molecular structure of **11** (ellipsoids are drawn at 50% probability). Hydrogen atoms are omitted for clarity. Selected interatomic distances [pm] and angles [°]: Co1–C1 203.4(2), Co1–C2 205.1(2), Co1–C3 204.9(2), Co1–C4 202.3(2), Co1–B2 214.2(2), Co1–B11 216.0(2), S1–B2 187.4(2), S1–C5 183.0(2), S2–B11 186.5(2), S2–C14 182.6(2), B2-S1-C5 103.91(9), B11-S2-C14 104.7(1).

can be used for linking these building blocks to a peptide through amide bond formation.

Thus, compound **6** was treated with ethyl bromide to give **11**. An increase in water solubility can be achieved by the introduction of a monosaccharide unit, such as isopropylidene-protected α-D-6-deoxygalactopyranose. Therefore, **6** was treated with protected α-D-6-deoxygalactopyranosyl triflate R^2OTf (**13**)[18] to give **14**.

Alkaline cleavage of the ethyl ester groups in **11** and **14** gave the final building blocks **12** and **15**, suitable for incorporation into peptides. Once **15** was incorporated into a peptide, the removal of the isopropylidene protecting groups (and thus an increase in water solubility) was achieved by treatment with trifluoroacetic acid (TFA), a common reagent for the cleavage of a peptide from a resin in the final stage of Fmoc-based solid-phase peptide synthesis (Fmoc-SPPS). The modification of the mercapto group in **6** increases the stability of the derivatives **11**–**15**: the compounds can be handled in air as solids and in solution without any problems. Compounds **11**–**15** were obtained as deep red powders. Single crystals suitable for X-ray crystallography could be obtained from a solution of **11** in CH_2Cl_2/MeCN (9:1, *v*/*v*) by slow solvent evaporation. The molecular structure of **11** is shown in Figure 1.

The final building blocks **12** and **15** were successfully subjected to an Fmoc-SPPS procedure.

Peptide synthesis

In previous studies, the modification of Lys^4 in NPY and in [F^7,P^{34}]-NPY (Table 1), the Y_1-receptor-selective derivative of neuropeptide Y, was found not to affect tumor selectivity or receptor internalization.[10a,e] Thus, only minor changes in the binding affinity were observed after incorporation of an *ortho*-carboranyl propionic acid unit into the peptide.[10a]

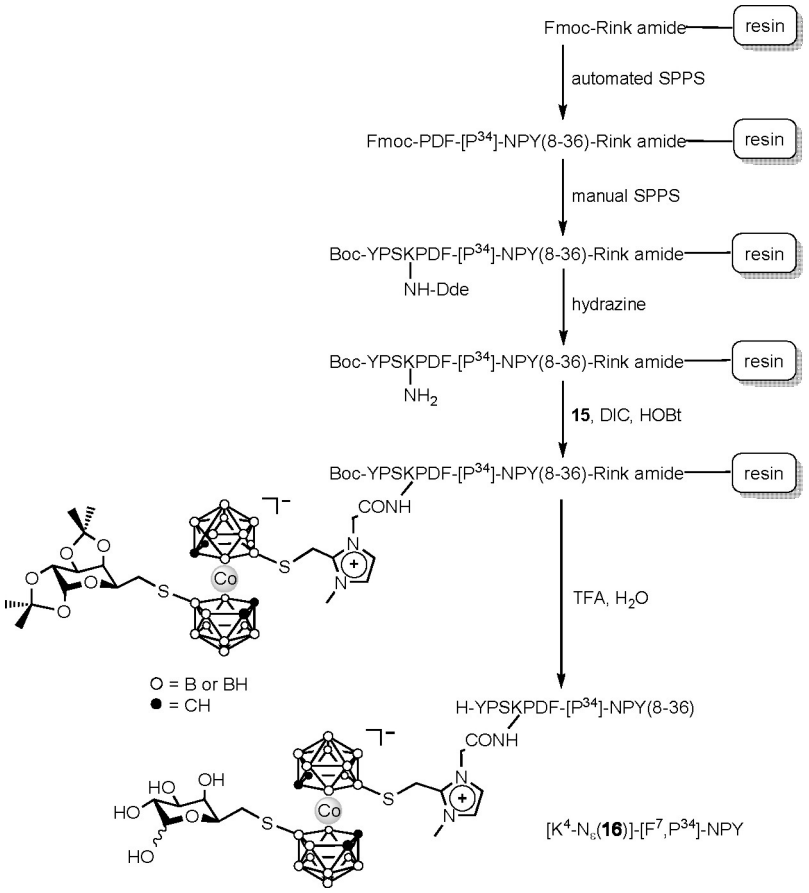
Table 1. Peptide sequences.

Peptide	Sequence
NPY	YPSKPDNPGEDAPAEDLARYYSALRHYINLITRQRY-NH_2
[F^7,P^{34}]-NPY	YPSKPD**F**PGEDAPAEDLARYYSALRHYINLITR**P**RY-NH_2

Furthermore, the L-lysine residue provides an N_ε-amino group that is separated by four carbon atoms from the peptide backbone and suitable for amide bond formation. We successfully applied a previously elaborated protocol[10b] using Fmoc-Lys(Dde)-OH bearing a 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) protecting group, which is orthogonal to the 9-fluorenylmethoxycarbonyl/*tert*-butyl (Fmoc/*tert*-Bu) protection strategy used. This amino acid was introduced in position 4, and after completion of the peptide synthesis the Dde protecting group could be removed selectively with hydrazine. Therefore, it was necessary to use the Boc-protected amino acid (Boc-Tyr-OH) in position 1. After Dde removal, compound **12** or **15** was coupled to the free N_ε-amino group of Lys^4 (Scheme 5). After cleavage from the Rink amide resin, the cobalt bis(dicarbollide)-conjugated peptide was purified by HPLC and analyzed by ESI mass spectrometry (Figure 2). This method also provides unambiguous confirmation of the removal of both isopropylidene groups from the galactose moiety to give the peptide [K^4-N_ε(**16**)]-[F^7,P^{34}]-NPY, thus confirming that building block **15** is fully compatible with the protocols of state-of-the-art Fmoc-SPPS.

Receptor activation studies

IP accumulation assays investigating the ability of native and boron-modified peptides to activate hY_1R and hY_2R revealed that [K^4-N_ε(**12**)]-[F^7,P^{34}]-NPY and [K^4-N_ε(**16**)]-[F^7,P^{34}]-NPY both activate hY_1R at similar concentrations to those of NPY and [F^7,P^{34}]-NPY, with EC_{50} values in a low nanomolar range (Table 2). On the other hand, hY_2R was activated at peptide concentrations even higher than those required in the case of [F^7,P^{34}]-NPY. Therefore, the selective receptor activation profile of the modified peptides was maintained for the peptides containing the charge-compensated metallacarborane building blocks.



Scheme 5. SPPS of Lys[4]-modified [F[7],P[34]]-NPY by the Dde protecting group strategy, demonstrated with compound **15**: synthesis of [F[7],P[34]]-NPY(5-36) by automated SPPS; manual coupling of Fmoc-K(Dde)-OH, Fmoc-S(*t*Bu)-OH, Fmoc-P-OH, and Boc-Y(*t*Bu)-OH; Dde cleavage with hydrazine; coupling of compound **15** with diisopropyl carbodiimide (DIC) and hydroxybenzotriazole (HOBt); cleavage of all protecting groups and the peptide from the resin with 5% (*v*/*v*) H_2O in TFA to give [K[4]-N$_\varepsilon$(**16**)]-[F[7],P[34]]-NPY.

Table 2. Receptor activation profiles.

Peptide	EC_{50} [nM] ($pEC_{50} \pm$ SEM)	
	hY_1R	hY_2R
NPY	2.5 (8.59 ± 0.05)	0.4 (9.44 ± 0.07)
[F[7],P[34]]-NPY	2.1 (8.67 ± 0.07)	81 (7.09 ± 0.09)
[K[4]-N$_\varepsilon$(**12**)]-[F[7],P[34]]-NPY	3.8 (8.42 ± 0.28)	341 (6.47 ± 0.28)
[K[4]-N$_\varepsilon$(**16**)]-[F[7],P[34]]-NPY	1.9 (8.473 ± 0.18)	138 (6.86 ± 0.27)

Receptor internalization

Internalization studies were performed on cells stably expressing hY_1R or hY_2R N-terminally fused to EYFP (enhanced yellow fluorescent protein) to confirm selective peptide uptake into these cells indirectly (Figure 3). Receptor localization was investigated prior to stimulation and 60 min after stimulation of cells with 1 μM peptide solution. Before stimulation, hY_1R and hY_2R were mainly located in the cell membrane. As a result of intracellular protein turnover, some receptors were detected inside the cytoplasm. For hY_1R, both the universal ligand NPY and the Y_1-receptor-selective [F[7],P[34]]-NPY promoted receptor internalization, resulting in receptors that were no longer located in the plasma membrane but in vesicles inside the cytoplasm. [K[4]-N$_\varepsilon$(**12**)]-[F[7],P[34]]-NPY and [K[4]-N$_\varepsilon$(**16**)]-[F[7],P[34]]-NPY were also able to induce internalization of hY_1 receptors. In contrast, hY_2R internalization only occurred after stimulation with the universal ligand NPY; stimulation with [F[7],P[34]]-NPY and its derivatives containing the charge-compensated metallacarborane building blocks did not affect receptor localization relative to the unstimulated state.

Comments on toxicity

Boron neutron capture is not a very efficient process, and the required amounts of 10^9 ^{10}B atoms (or 30 μg ^{10}B per g of tumor tissue) are high in relation to other tumor agents. The stated amount is considered the minimum concentration needed to generate three to seven α particles per cell to induce lethal damage.[8b] Thus, the majority of the boron cages indeed remain undamaged after the neutron treatment, but their fate within the tumor cell is unknown. Although cobalt bis(dicarbollide) **1** is presumed to be a very stable anion under laboratory conditions, the behavior of **1** (and its derivatives) in cells is unclear. In particular, lysosomal digestion could (in the worst case) cause complete degradation of the boron cages. For cobalt bis(dicarbollide) this process would result in the libera-

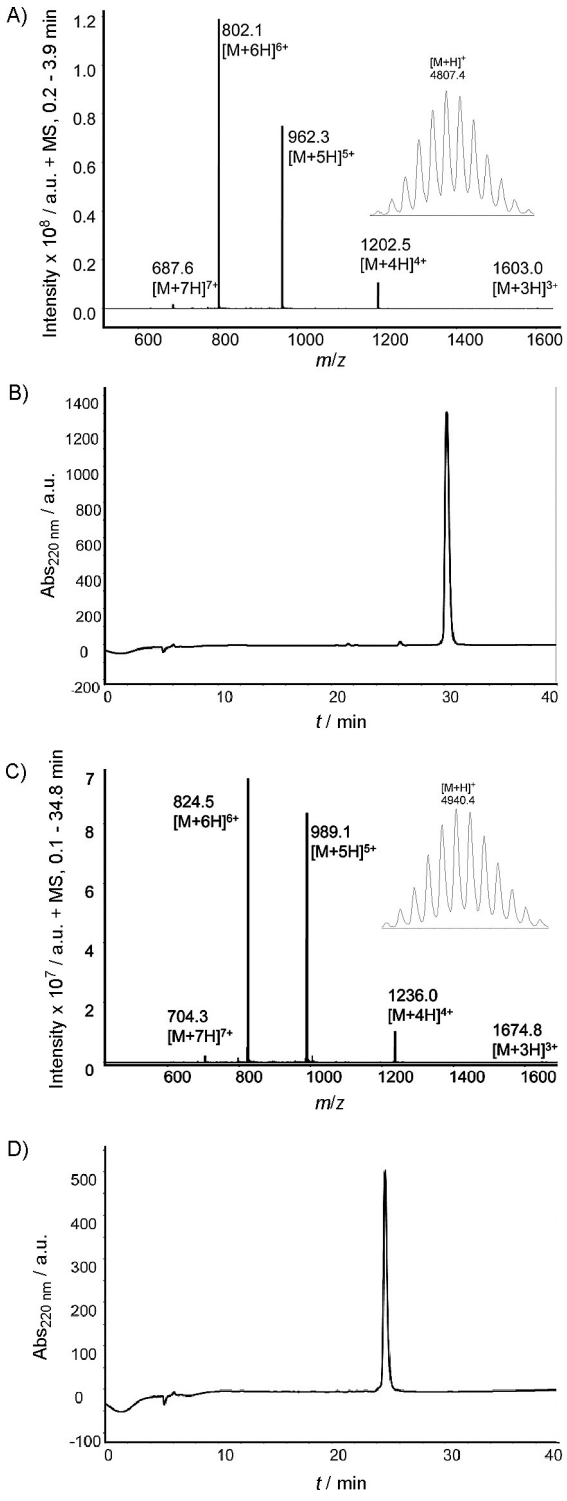


Figure 2. Analytical data for [K^4-N$_\varepsilon$(**12**)]-[F^7,P^{34}]-NPY. A) ESI mass spectrum displaying the following peaks: $[M+H]^+ = 4807.4$ (with isotopic pattern), $[M+4H]^{4+} = 1202.5$, $[M+5H]^{5+} = 962.3$, $[M+6H]^{6+} = 802.1$, resulting experimental mass $M_{exp} = 4806.3$ Da (calculated mass $M_{calcd} = 4807$ Da, $C_{208}H_{322}B_{18}CoN_{55}O_{56}S_2$). B) RP-HPLC chromatogram ($t_R = 30.5$ min). Analytical data for [K^4-N$_\varepsilon$(**16**)]-[F^7,P^{34}]-NPY. C) ESI mass spectrum displaying the following peaks: $[M+H]^+ = 4940.4$ (with isotopic pattern), $[M+4H]^{4+} = 1236.0$, $[M+5H]^{5+} = 989.1$, $[M+6H]^{6+} = 824.5$, resulting experimental mass $M_{exp} = 4940.5$ Da (calculated mass $M_{calcd} = 4941$ Da, $C_{212}H_{328}B_{18}CoN_{55}O_{61}S_2$). D) RP-HPLC chromatogram ($t_R = 24.8$ min).

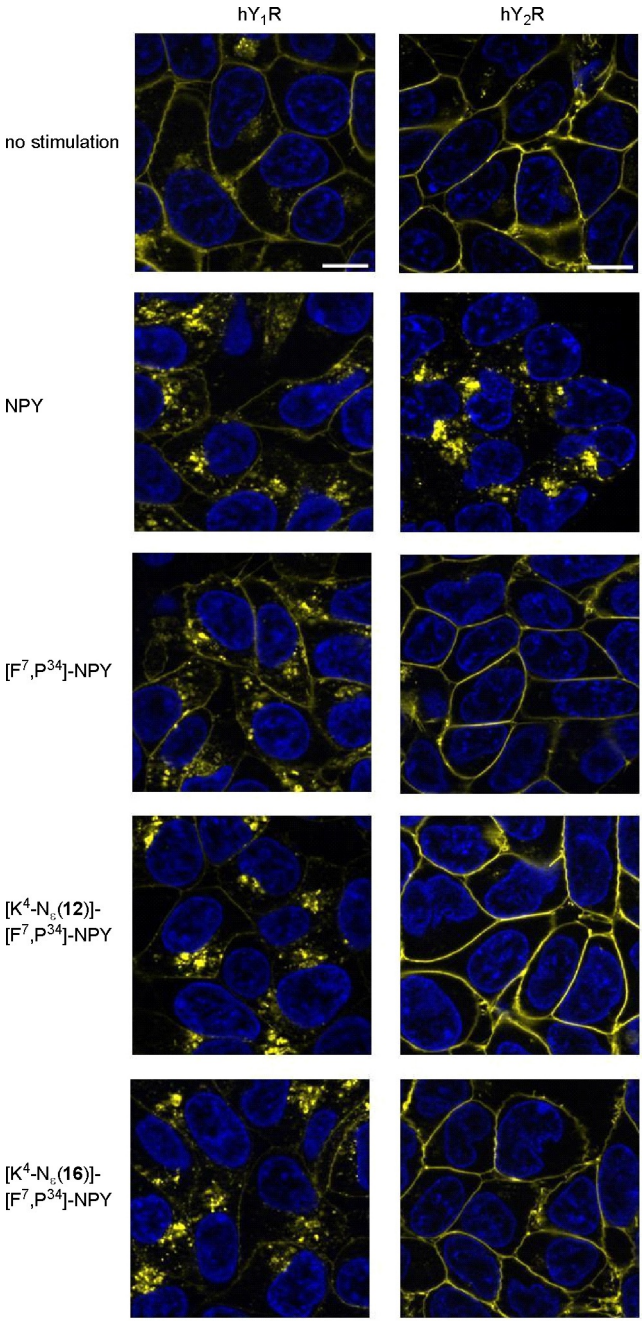


Figure 3. Receptor internalization studies of metallacarborane-containing [F^7,P^{34}]-NPY conjugates [K^4-N$_\varepsilon$(**12**)]-[F^7,P^{34}]-NPY and [K^4-N$_\varepsilon$(**16**)]-[F^7,P^{34}]-NPY in comparison with NPY and [F^7,P^{34}]-NPY on HEK293 cells stably expressing either hY$_1$R or hY$_2$R fused to EYFP (yellow). Cell nuclei were stained with Hoechst 33342 (blue). Cells were stimulated with 1 μM of peptides for 1 h. Representative pictures of three independent experiments are shown. Scale bar: 10 μm.

tion of $Co^{2+/3+}$ (besides other potentially toxic boron species), and the question arises of whether cobalt exerts a pronounced toxicity towards the patient or not. Although derivatives of **1** have been proposed as boron-delivering agents, this issue has, to the best of our knowledge, never been discussed in detail. In an approximate estimation we wish to demonstrate that the dose of cobalt is unlikely to reach a severe lethal limit.

The impact of cobalt on the human body in the form of $CoCl_2$ or $CoSO_4$ has been investigated in previous studies; these simple inorganic salts are traditionally used to treat anemia[19a,b] and are well-established chemical inducers of hypoxia-like responses such as erythropoiesis or angiogenesis.[19c] The oral doses of cobalt salts that are tolerated by particular organisms appear to depend strongly on the animal model. Thus, in studies with various mouse types, no mortality or toxic effects were reported up to oral doses of 40 mg kg^{-1} (of $CoCl_2$) over a period of four months,[19d] whereas initial symptoms of organ damage were observed in male Fischer 344 rats upon a single application of an oral dose of 33.3 mg kg^{-1}.[19e] The intravenous administration of cobalt salts proved to give more consistent values throughout various animal models, because it bypassed the gastrointestinal tract. Although, in one reported case, $CoSO_4$ in a very high cumulative intravenous dose (>21 days, 110 mg kg^{-1}) induced heart failure in rats,[19f] intravenous doses of 4 mg kg^{-1} ($CoCl_2$ in male Fischer 344 rats, single application)[19e] or 5 mg kg^{-1} ($CoSO_4$ in canine model, exposure for several months) were extremely well tolerated.[19g] The long-term intravenous doses of cobalt salts for which no severe malfunctions of organs are reported amount to ca. 5 mg kg^{-1} of cobalt(II) in various animal models. For risk assessment of BNCT agents, we find reference to intravenous toxicity more suitable. Because no studies providing intravenous doses for humans are available, to the best of our knowledge, we assumed a low value of 1 mg kg^{-1} of Co as a suitable value for estimations. In a patient of average weight (75 kg) the total load of cobalt that would conveniently be tolerated amounts to 75 mg. On the assumption that this amount is generated from anion **1** (^{10}B-enriched: $C_4H_{22}{}^{10}B_{18}Co$), the content of ^{10}B in the patient (ideally located in tumor tissue including metastases) would be ca. 229 mg. With the therapeutically accepted value of ca. 30 μg ^{10}B per g tumor tissue the stated amount of Co (75 mg or 1 mg kg^{-1} body mass) would (hypothetically) be generated from ca. 7.633 kg of tumor tissue. In practice, however, the masses of tumor tissue including metastases only cover the range of a few grams, and we are, therefore, confident that BNCT agents derived from anion **1** do not liberate cobalt in potentially harmful concentrations.

Conclusions

A high-yielding synthesis of compound **6**, a new imidazolium-based charge-compensated metallacarborane for incorporation into peptides, has been developed. Compound **6** is versatile and can readily be further modified, as shown here by ethyl protection and deoxygalactosylation of the mercapto group; however, other moieties of biochemical importance—poly(ethylene glycol) (PEG), for example—could also be included by means of nucleophilic substitution reactions. The zwitterionic building blocks **12** and **15** could successfully be conjugated to [F^7,P^{34}]-NPY, the Y_1-receptor-selective derivative of neuropeptide Y, while avoiding the presence of countercations. Furthermore, in the final step in the established Fmoc-SPPS protocol, treatment with TFA resulted in removal of the isopropylidene groups in **15** with formation of a hydrophilic deoxygalactosyl moiety. Receptor activation studies of the new metallacarborane-modified peptides showed the response of hY_1 receptor types to be in a very similar concentration range to that seen with [F^7,P^{34}]-NPY, whereas the response of hY_2 receptors was observed at concentrations significantly higher than in the case of [F^7,P^{34}]-NPY, thus indicating even better selectivity of the new peptides for the hY_1 receptor. Receptor internalization occurred in the same concentration range as observed for [F^7,P^{34}]-NPY. These observations indicate that the conjugated peptides [K^4-N$_\varepsilon$(**12**)]-[F^7,P^{34}]-NPY and [K^4-N$_\varepsilon$(**16**)]-[F^7,P^{34}]-NPY are promising candidates for further boron-accumulation studies.

Experimental Section

Chemical syntheses: All chemical reactions were carried out under ambient conditions, except for reactions involving **1**, **4**, **5**, or **6** either as a starting material or product; these were carried out under nitrogen. Solvents were purified and distilled under nitrogen prior to use.[20] All other chemicals were used as purchased. Compound **1** was prepared according to literature procedures, starting from 1,2-dicarba-*closo*-dodecaborane(12), $C_2B_{10}H_{12}$.[1,21] Compounds **1** and **5** were prepared as tetramethylammonium salts.

TLC was performed on precoated plates (0.25 mm, silica gel 60 F_{254}). Derivatives of **1** were visible on the plate without any treatment, due to the intense color of the compounds. Other compounds were visualized with molybdophosphoric acid in propan-2-ol. Column chromatography was carried out with silica gel (100–200 mesh). The ^{1}H, ^{11}B, and ^{13}C NMR spectra were recorded with a Bruker AVANCE DRX 400 spectrometer. The chemical shifts of ^{1}H, ^{11}B, and ^{13}C are reported in parts per million at 400.13, 128.38, and 100.63 MHz, respectively, with tetramethylsilane as internal standard and referencing to the unified scale.[22] Substituted boron atoms could be assigned by comparison of ^{11}B and ^{11}B{^{1}H} NMR spectra. FTIR spectra were recorded with a PerkinElmer system 2000 FTIR spectrometer, scanning between 400 and 4000 cm^{-1}, and using KBr disks. Mass spectra were recorded with a VG Analytics ZAB HSQ system with electrospray injection. Elemental analyses were carried out with a Hereaus VARIO EL oven. The melting points were measured in sealed tubes. The syntheses of the known compounds **1**, **4**, **5**, and **9** are not reported in detail; only modifications of the cited procedures are given, and analysis is limited to the most characteristic NMR data and the melting point.

Tetramethylammonium 3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate)(1−) (1): Compound **1** was prepared as described in the literature[1] and precipitated as the tetramethylammonium salt from aqueous solution; golden, plate-like crystals. Yield: 65% (lit. 70%); m.p. 292–293 °C (lit. 290–293 °C); ^{1}H NMR (CD_3CN): δ = 0.9–3.5 (several br s and m, 18H; B*H*), 3.10 (s, 12H; N(CH_3)$_4$), 3.89 ppm (s, 4H; cage C*H*); ^{11}B{^{1}H} NMR (CD_3CN): δ = −22.9, −17.5, −6.5, −5.8, 1.1, 6.0 ppm.

μ-8,8′-Dithioformato-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate)(1−) (4): Compound **4** was synthesized according to the literature.[16] The silica pad filtration was performed with $CHCl_3$ instead of CH_2Cl_2, resulting in a purer product; bright orange powder. Yield: 68% (lit. 70%); m.p. 301–302 °C (lit. 302–304 °C); ^{1}H NMR ($CDCl_3$): δ = 0.9–3.5 (several br s and m, 16H; B*H*), 3.81 (s, 4H; cage C*H*), 10.83 ppm (s, 1H; S_2CH); ^{11}B{^{1}H} NMR ($CDCl_3$): δ = −22.3, −16.6, −9.2, −3.2, 2.4, 11.6 ppm.

Tetramethylammonium 8,8′-dimercapto-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate)(1−) (5): Com-

pound **5** was prepared according to the literature[16] and precipitated as the tetramethylammonium salt from aqueous solution; red powder. Yield: quantitative (lit. quantitative); m.p. 265–267 °C (decomp.); ^{1}H NMR ([D$_6$]acetone): $\delta = 0.42$ (m, 2H; S*H*), 0.9–3.5 (several br s and m, 16H; B*H*), 3.46 (s, 12H; N(C*H*$_3$)$_4$), 4.40 ppm (s, 4H; cage C*H*); ^{11}B{^{1}H} NMR ([D$_6$]acetone): $\delta = -24.2$, −17.8, −4.3, 0.5, 10.1 ppm.

8-[1-(Ethoxycarbonylmethyl)-3-methyl-2-imidazolium methylthio]-8′-mercapto-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate) (1−) (6): Compound **4** (400 mg, 1.00 mmol) was dissolved in degassed EtOH (50 mL), and one drop of HCl (aqueous, concentrated) was added. The mixture was heated to 70 °C for 5 min (longer heating should be avoided, to prevent complete reaction to afford the bismercapto anion in compound **5**) and allowed to cool to ambient temperature. Then **7** (253 mg, 1.00 mmol) was added as a powder. The mixture was maintained at 70 °C for 24 h, and the degree of conversion was monitored by TLC with EtOH as eluent. Column chromatography was performed with CH_2Cl_2; the elution of **6** was indicated by the appearance of a broad orange band. The solvent was immediately removed under reduced pressure to give **6** as a red, crusty material, soluble in CH_2Cl_2, MeCN, or EtOH. $R_f = 0.2$ (in EtOH). Yield: 513 mg (90%); m.p. 111–114 °C; ^{1}H NMR (CD$_3$CN): $\delta = 0.59$ (m, 1H; S*H*), 0.9–3.5 (several br s and m, 16H; B*H*), 1.28 (t, ^3J(H,H) = 7.0 Hz, 3H; OCH$_2$C*H*$_3$), 3.78 (s, 3H; NC*H*$_3$), 3.80 (s, 2H; SC*H*$_2$CN$_2$), 4.24 (q, ^3J(H,H) = 7.0 Hz, 2H; OC*H*$_2$CH$_3$), 4.41 (s, 4H; cage C*H*), 4.94 (s, 2H; C*H*$_2$COOEt), 7.28 ppm (s, 2H; aryl C*H*); ^{11}B{^{1}H} NMR (CD$_3$CN): $\delta =$ −24.1, −16.9, −7.5, −4.8, −4.0, −0.5, 0.7, 10.8 (*B*S), 12.0 ppm (*B*S); ^{13}C{^{1}H} NMR (CD$_3$CN, APT): $\delta = 13.4$ (OCH$_2$*C*H$_3$), 23.3 (S*C*H$_2$CN$_2$), 35.4 (NCH$_3$), 49.1 (*C*H$_2$COOEt), 53.8 (cage *C*H), 55.9 (cage *C*H), 62.6 (O*C*H$_2$CH$_3$), 122.8 (aryl *C*H), 122.9 (aryl *C*H), 147.1 (SCH$_2$*C*N$_2$), 165.9 ppm (CH$_2$*C*OOEt); IR: $\tilde{\nu} = 3060$ (w, aryl C–H), 2961 (m, alkyl C–H), 2568 (s, B–H), 1751 (m, C=O), 1262 (m, C–O), 1101 (m), 1019 (m), 801 cm^{-1} (m); MS (ESI−): 568.2 [*M*−H]$^-$, 604.1 [*M*+Cl]$^-$ (100%); MS (ESI+): 570.2 [*M*+H]$^+$ (100%); elemental analysis calcd (%) for C$_{13}$H$_{35}$B$_{18}$CoN$_2$O$_2$S$_2$: N 4.92, C 27.43, H 6.21; found N 5.13, C 27.28, H 6.03.

2-(Chloromethyl)-1-(ethoxycarbonylmethyl)-3-methylimidazolium chloride (7): Compound **8** (1.00 g, 4.26 mmol) was dissolved in MeCN (50 mL). Thionyl chloride ($SOCl_2$, 1.22 g, 0.75 mL, 2.5 equiv) was added, and the solution was heated to reflux for 1 h. The solvent and excess $SOCl_2$ were removed under reduced pressure. The remaining orange oil was dissolved in MeCN (5–10 mL), and the product was precipitated with Et$_2$O. Filtration gave **7** as a colorless, crystalline material, soluble in MeCN. Yield: 810 mg (75%); m.p. 94–95 °C; ^{1}H NMR (CD$_3$CN): $\delta = 1.27$ (t, ^3J(H,H) = 7.2 Hz, 3H; OCH$_2$C*H*$_3$), 3.99 (s, 3H; NC*H*$_3$), 4.40 (q, ^3J(H,H) = 7.2 Hz, 2H; OC*H*$_2$CH$_3$), 5.32 (s, 2H; C*H*$_2$COOEt), 5.49 (s, 2H; C*H*$_2$Cl), 7.69 (s, 1H; aryl C*H*), 7.83 ppm (s, 1H; aryl C*H*); ^{13}C{^{1}H} NMR (CD$_3$CN, APT): $\delta =$ 13.4 (OCH$_2$*C*H$_3$), 30.4 (*C*H$_2$Cl), 35.7 (NCH$_3$), 49.6 (*C*H$_2$COOEt), 62.5 (O*C*H$_2$CH$_3$), 124.2 (aryl *C*H), 124.2 (aryl *C*H), 143.1 (N$_2$*C*CH$_2$Cl), 166.2 ppm (CH$_2$*C*OOEt); IR: $\tilde{\nu} = 3139$ (m, aryl C–H), 3075 (m, aryl C–H), 2987 (m, alkyl C–H), 2913 (m, alkyl C–H), 2859 (m, alkyl C–H), 1753 (s, C=O), 1584 (w), 1536 (m), 1435 (m), 1262 (m, C–O), 1160 (m), 1018 (m), 802 cm^{-1} (m); MS (ESI+): 217.0 [*M*−Cl]$^+$ (100%); elemental analysis calcd (%) for C$_9$H$_{14}$Cl$_2$N$_2$O$_2$: N 11.07, C 42.70, H 5.59; found N 11.30, C 42.53, H 5.80.

1-(Ethoxycarbonylmethyl)-2-(hydroxymethyl)-3-methylimidazolium chloride (8): Compound **9** (1.00 g, 8.92 mmol) was dissolved in EtOH (50 mL), and ethyl chloroacetate (ClCH$_2$COOEt, 8.70 g, 7.60 mL, 8 equiv) was added. The solution was heated to 60 °C for 12 h. The solvent and excess ethyl chloroacetate were removed under reduced pressure. The remaining material was subjected to silica pad filtration (ca. 4 cm of silica gel) and eluted with ethanol (TLC monitoring, $R_f = 0.50$ in EtOH). The solvent was removed under reduced pressure. Compound **8** was obtained as a colorless oil, soluble in MeCN and EtOH. Yield: 1.68 g (80%). ^{1}H NMR (CD$_3$CN): $\delta = 1.27$ (t, ^3J(H,H) = 7.2 Hz, 3H; OCH$_2$C*H*$_3$), 3.93 (s, 3H; NC*H*$_3$), 4.23 (q, ^3J(H,H) = 7.2 Hz, 2H; OC*H*$_2$CH$_3$), 4.73 (s, 2H; C*H*$_2$OH), 5.33 (s, 2H; C*H*$_2$COOEt), 6.90 (br s, 1H; CH$_2$O*H*), 7.36 (s, 1H; aryl C*H*), 7.39 ppm (s, 1H; aryl C*H*); ^{13}C{^{1}H} NMR (CD$_3$CN, APT): $\delta = 13.4$ (OCH$_2$*C*H$_3$), 35.4 (NCH$_3$), 49.5 (*C*H$_2$COOEt), 51.2 (*C*H$_2$OH), 62.5 (O*C*H$_2$CH$_3$), 122.7 (aryl *C*H), 122.8 (aryl *C*H), 147.2 (N$_2$*C*CH$_2$OH), 166.6 ppm (CH$_2$*C*OOEt); IR: $\tilde{\nu} = 3224$ (s, O–H), 3075 (m, aryl C–H), 2960 (m, alkyl C–H), 1745 (s, C=O), 1636 (w), 1540 (m), 1376 (w), 1225 (s, C–O), 1165 (m), 1095 (m), 1034 (s), 803 cm^{-1} (m); MS (ESI+): 199.0 [*M*−Cl]$^+$ (100%), 433.0 [2*M*−Cl]$^+$; elemental analysis calcd (%) for C$_9$H$_{15}$ClN$_2$O$_3$: N 11.94, C 46.05, H 6.45; found N 11.80, C 46.23, H 6.53.

2-(Hydroxymethyl)-1-methylimidazole (9): Compound **9** was obtained according to a modified literature procedure.[17] The remaining paraformaldehyde was removed by filtration, and compound **9** was crystallized from the solution by slow addition of Et$_2$O. Yield: 69% (lit. 71%); m.p. 114–115 °C; ^{1}H NMR (CD$_3$CN): $\delta = 3.65$ (s, 3H; NC*H*$_3$), 4.54 (s, 2H; C*H*$_2$OH), 5.10 (br s, 1H; CH$_2$O*H*), 6.77 (s, 1H; aryl C*H*), 6.92 ppm (s, 1H; aryl C*H*); ^{13}C{^{1}H} NMR (CD$_3$CN): $\delta = 32.2$ (NCH$_3$), 55.5 (*C*H$_2$OH), 121.8 (aryl *C*H), 126.1 (aryl *C*H), 148.0 ppm (N$_2$*C*CH$_2$OH).

8-[1-(Ethoxycarbonylmethyl)-3-methyl-2-imidazolium methylthio]-8′-ethylthio-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate) (1−) (11): Compound **6** (500 mg, 0.88 mmol) was dissolved in MeCN (25 mL, anhydrous and degassed). Potassium carbonate (K_2CO_3, 245 mg, 2 equiv) and bromoethane (EtBr, 480 mg, 0.33 mL, 5 equiv) were added. The mixture was stirred for 12 h and then diluted with CH_2Cl_2 (200 mL) and water (150 mL). HCl (20 mL, aqueous, diluted) was added, initially only dropwise, due to intense bubbling. After phase separation, the organic phase was dried over $MgSO_4$, and the solvent was removed under reduced pressure. Silica pad filtration (ca. 7 cm of silica gel) with CH_2Cl_2/MeCN (9:1, *v/v*) afforded one broad orange band. Crystals suitable for X-ray crystallography could be obtained by allowing the eluate to stand for 3 d. Solvent removal gave **11** as a dark red powder, slightly soluble in CH_2Cl_2 and MeCN and soluble in acetone. Yield: 472 mg (90%); m.p. 227–229 °C; $R_f = 0.50$ in EtOH. ^{1}H NMR (CD$_3$CN): $\delta = 0.9$–3.5 (several br s and m, 16H; B*H*), 1.12 (t, ^3J(H,H) = 7.6 Hz, 3H; SCH$_2$C*H*$_3$), 1.28 (t, ^3J(H,H) = 7.2 Hz, 3H; OCH$_2$C*H*$_3$), 2.40 (m, 2H; SC*H*$_2$CH$_3$), 3.77 (s, 3H; NC*H*$_3$), 3.79 (s, 2H; SC*H*$_2$CN$_2$), 4.23 (q, ^3J(H,H) = 7.2 Hz, 2H; OC*H*$_2$CH$_3$), 4.38 (s, 2H; cage C*H*), 4.40 (s, 2H; cage C*H*), 4.93 (s, 2H; C*H*$_2$COOEt), 7.27 ppm (s, 2H; aryl C*H*); ^{11}B{^{1}H} NMR (CD$_3$CN): $\delta = -23.9$, −17.2, −7.7, −6.1, −5.0, −0.7, 0.6, 9.4 (*B*S), 13.6 ppm (*B*S); ^{13}C{^{1}H} NMR (CD$_3$CN): $\delta =$ 13.4 (OCH$_2$*C*H$_3$), 16.6 (SCH$_2$*C*H$_3$), 23.0 (SCH$_2$*C*N$_2$), 27.3 (SCH$_2$*C*H$_3$), 35.4 (NCH$_3$), 49.0 (*C*H$_2$COOEt), 53.8 (cage *C*H), 55.8 (cage *C*H), 62.6 (O*C*H$_2$CH$_3$), 122.8 (aryl *C*H), 122.9 (aryl *C*H), 147.2 (SCH$_2$*C*N$_2$), 166.0 ppm (CH$_2$*C*OOEt); IR: $\tilde{\nu} = 3110$ (w, cage C–H), 3030 (w, aryl C–H), 2959 (m, alkyl C–H), 2930 (m, alkyl C–H), 2554 (s, B–H), 1735 (s, C=O), 1585 (m), 1534 (m), 1444 (m), 1263 (m, C–O), 1100 (m), 1019 (m), 843 cm^{-1} (m); MS (ESI−): 596.1 [*M*−H]$^-$, 632.1 [*M*+Cl]$^-$ (100%); elemental analysis calcd (%) for C$_{15}$H$_{39}$B$_{18}$CoN$_2$O$_2$S$_2$: N 4.69, C 30.17, H 6.60; found N 4.73, C 29.95, H 6.85.

8-[1-(Carboxymethyl)-3-methyl-2-imidazolium methylthio]-8′-ethylthio-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate) (1−) (12): Compound **11** (400 mg, 0.67 mmol) was dissolved in MeOH (20 mL), and sodium hydroxide

(NaOH, 80 mg, 3 equiv) was added. The solution was heated and maintained at 60 °C for 30 min. Completion was indicated by TLC with EtOH as an eluent (R_f = 0.15 in EtOH). The mixture was diluted with CH_2Cl_2 (200 mL) and water (150 mL). HCl (20 mL, aqueous, dilute) was added. After phase separation, the organic phase was dried over $MgSO_4$. Solvent removal gave **12** as a red powder, slightly soluble in CH_2Cl_2 and soluble in MeCN and acetone. Yield: 378 mg (quantitative); m.p. 138–140 °C; ^{1}H NMR (CD_3CN): δ = 0.9–3.5 (several brs and m, 16H; B*H*), 1.13 (t, ^3J(H,H) = 7.6 Hz, 3H; SCH_2CH_3), 2.39 (m, 2H; SCH_2CH_3), 3.77 (s, 5H; NCH_3 and SCH_2CN_2), 4.37 (s, 2H; cage C*H*), 4.41 (s, 2H; cage C*H*), 4.92 (s, 2H; CH_2COOH), 7.28 ppm (s, 2H; aryl C*H*); ^{11}B{^{1}H} NMR (CD_3CN): δ = −23.8, −17.1, −7.6, −6.1, −5.0, −0.6, 0.6, 9.4 (*BS*), 13.6 ppm (*BS*); ^{13}C{^{1}H} NMR (CD_3CN): δ = 16.7 (SCH_2CH_3), 23.1 (SCH_2CN_2), 27.3 (SCH_2CH_3), 35.5 (NCH_3), 49.0 (CH_2COOH), 54.0 (cage C*H*), 55.8 (cage C*H*), 122.8 (aryl C*H*), 122.9 (aryl C*H*), 146.1 (SCH_2CN_2), 166.0 ppm (CH_2COOH); IR: $\tilde{\nu}$ = 3145 (w, cage C–H), 3040 (w, aryl C–H), 2922 (m, alkyl C–H), 2851 (m, alkyl C–H), 2566 (s, B–H), 1737 (s, C=O), 1587 (m), 1530 (m), 1445 (m), 1262 (m, C–O), 1102 (m), 989 (m), 841 cm^{-1} (m); MS (ESI−): 568.2 $[M-H]^-$ (100%); MS (ESI+): 570.3 $[M+H]^+$ (80%); elemental analysis calcd (%) for $C_{13}H_{35}B_{18}CoN_2O_2S_2$: N 4.92, C 27.43, H 6.21; found N 5.20, C 27.68, H 6.35.

1,2:3,4-Di-*O*-isopropylidene-6-*O*-trifluoromethanesulfonyl-α-D-galactopyranose (13): Compound **13** was synthesized from commercially available 1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranose according to the literature.[18] Yield: 93% (lit. 95%); m.p. 45–46 °C (lit. 47 °C); ^{1}H NMR ($CDCl_3$): δ = 1.34 (s, 6H; $C(CH_3)_2$), 1.45 (s, 3H; $C(CH_3)_2$), 1.53 (s, 3H; $C(CH_3)_2$), 4.15 (m, 1H; H-5), 4.25 (m, 1H; H-4), 4.37 (m, 1H; H-2), 4.57-4.67 (m, 3H; H-3, H-6, H-6′), 5.54 ppm (d, ^3J(H,H) = 4.8 Hz, 1H; H-1); ^{13}C{^{1}H} NMR ($CDCl_3$, APT): δ = 24.3 ($C(CH_3)_2$), 24.8 ($C(CH_3)_2$), 25.8 ($C(CH_3)_2$), 25.9 ($C(CH_3)_2$), 66.1 (C-5), 70.2 (C-2, C-3, or C-4), 70.4 (C-2, C-3, or C-4), 70.6 (C-2, C-3, or C-4), 74.7 (C-6), 96.1 (C-1), 109.1 ($C(CH_3)_2$), 110.1 ($C(CH_3)_2$), 118.6 ppm (q, ^1J(C,F) = 319.9 Hz, CF_3).

8-[1-(Ethoxycarbonylmethyl)-3-methyl-2-imidazolium methylthio]-8′-(1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-D-galacto-pyranosylthio)-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate) (1−) (14): Compound **6** (500 mg, 0.88 mmol) was dissolved in MeCN (25 mL, anhydrous and degassed). Potassium carbonate (K_2CO_3, 245 mg, 2 equiv) and **13** (396 mg, 1.15 equiv) were added. The mixture was stirred for 12 h and then diluted with CH_2Cl_2 (200 mL) and water (150 mL). HCl (20 mL, aqueous, diluted) was added slowly (intense bubbling). After phase separation, the organic phase was dried over $MgSO_4$; after filtration, the solvent was removed under reduced pressure. Column chromatography was performed with a continuous gradient change of the eluent (100% CH_2Cl_2 to CH_2Cl_2/MeCN 96:4, *v*/*v*) to afford one broad orange band (R_f = 0.10, CH_2Cl_2/MeCN 96:4, *v*/*v*). Solvent removal gave **14** as a dark red powder, soluble in CH_2Cl_2 and MeCN. Yield: 606 mg (85%); m.p. 145–147 °C; ^{1}H NMR (CD_3CN): δ = 0.9–3.5 (several brs and m, 16H; B*H*), 1.28 (m, 6H; $(CH_3)C(CH_3)$, OCH_2CH_3), 1.30 (s, 3H; $C(CH_3)_2$), 1.34 (s, 3H; $C(CH_3)_2$), 1.46 (s, 3H; $C(CH_3)_2$), 2.40 (m, 1H; H-6), 2.59 (m, 1H; H-6′), 3.67 (m, 1H; H-5), 3.77 (s, 3H; NCH_3), 3.79 (s, 2H; SCH_2CN_2), 4.20-4.30 (m, 4H; H-2, H-4, OCH_2CH_3), 4.40 (s, 4H; cage C*H*), 4.56 (d, ^3J(H,H) = 7.6 Hz, 1H; H-3), 4.93 (s, 2H; CH_2COOEt), 5.38 (d, ^3J(H,H) = 4.8 Hz, 1H; H-1), 7.28 ppm (s, 2H; aryl C*H*); ^{11}B{^{1}H} NMR (CD_3CN): δ = −23.8, −17.0, −7.5, −6.1, −5.1, −0.5, 0.5, 9.8 (*BS*), 13.5 ppm (*BS*); ^{13}C{^{1}H} NMR (CD_3CN, APT): δ = 13.4 (OCH_2CH_3), 23.1 (SCH_2CN_2), 23.8 ($C(CH_3)_2$), 24.2 ($C(CH_3)_2$), 25.3 ($C(CH_3)_2$), 25.4 ($C(CH_3)_2$), 32.6 (C-6), 35.4 (NCH_3), 49.0 (CH_2COOEt), 53.8 (cage C*H*), 55.7 (cage C*H*), 62.6 (OCH_2CH_3), 68.3 (C-2, C-3, C-4, or C-5), 70.4 (C-2, C-3, C-4, or C-5), 70.8 (C-2, C-3, C-4, or C-5), 71.2 (C-2, C-3, C-4, or C-5), 96.4 (C-1), 108.2 ($C(CH_3)_2$), 108.5 ($C(CH_3)_2$), 122.8 (aryl C*H*), 122.9 (aryl C*H*), 147.6 (SCH_2CN_2), 166.0 ppm (CH_2COOEt); IR: $\tilde{\nu}$ = 3144 (w, cage C–H), 3041 (w, aryl C–H), 2986 (m, alkyl C–H), 2936 (m, alkyl C–H), 2568 (s, B–H), 1751 (s, C=O), 1587 (m), 1532 (m), 1455 (m), 1381 (s), 1255 (s, C–O), 1214 (s, C–O), 1173 (m), 1100 (m), 1069 (m), 993 (s), 843 cm^{-1} (m); MS (ESI+): 834.3 $[M+Na]^+$ (100%); elemental analysis calcd (%) for $C_{25}H_{53}B_{18}CoN_2O_7S_2$: N 3.45, C 37.00, H 6.60; found N 3.60, C 36.83, H 6.50.

8-[1-(Carboxymethyl)-3-methyl-2-imidazolium methylthio]-8′-(1,2:3,4-di-*O*-isopropylidene-6-deoxy-α-D-galacto-pyranosylthio)-3,3′-*commo*-bis(undecahydro-1,2-dicarba-3-cobalta-*closo*-undecaborate) (1−) (15): Compound **14** (400 mg, 0.49 mmol) was dissolved in MeOH (20 mL), and sodium hydroxide (NaOH, 60 mg, 3 equiv) was added. The solution was heated and maintained at 60 °C for 30 min. Completion was indicated by TLC with EtOH as eluent (R_f = 0.15 in EtOH). The mixture was diluted with CH_2Cl_2 (200 mL) and water (150 mL). HCl (20 mL, aqueous, diluted) was added. After phase separation, the organic phase was dried over $MgSO_4$. Solvent removal gave **15** as a red powder, soluble in CH_2Cl_2 and MeCN. Yield: 382 mg (quantitative); m.p. ≈150 °C (decomp.); ^{1}H NMR (CD_3CN): δ = 0.9–3.5 (several brs and m, 16H; B*H*), 1.28 (s, 3H; $C(CH_3)_2$), 1.31 (s, 3H; $C(CH_3)_2$), 1.35 (s, 3H; $C(CH_3)_2$), 1.46 (s, 3H; $C(CH_3)_2$), 2.43 (m, 1H; H-6), 2.59 (m, 1H; H-6′), 3.67 (m, 1H; H-5), 3.77 (s, 3H; NCH_3), 3.79 (s, 2H; SCH_2CN_2), 4.26-4.31 (m, 2H; H-2, H-4), 4.40 (s, 4H; cage C*H*), 4.56 (d, ^3J(H,H) = 7.6 Hz, 1H; H-3), 4.93 (s, 2H; CH_2COOH), 5.38 (d, ^3J(H,H) = 4.7 Hz, 1H; H-1), 7.27 (s, 1H; aryl C*H*), 7.28 ppm (s, 1H; aryl C*H*); ^{11}B{^{1}H} NMR (CD_3CN): δ = −23.8, −17.0, −7.5, −6.1, −5.1, −0.4, 0.5, 9.7 (*BS*), 13.4 ppm (*BS*); ^{13}C{^{1}H} NMR (CD_3CN, APT): δ = 23.1 (SCH_2CN_2), 23.8 ($C(CH_3)_2$), 24.2 ($C(CH_3)_2$), 25.3 ($C(CH_3)_2$), 25.4 ($C(CH_3)_2$), 32.6 (C-6), 35.4 (NCH_3), 48.7 (CH_2COOH), 53.8 (cage C*H*), 55.7 (cage C*H*), 68.3 (C-2, C-3, C-4, or C-5), 70.4 (C-2, C-3, C-4, or C-5), 70.7 (C-2, C-3, C-4, or C-5), 71.2 (C-2, C-3, C-4, or C-5), 96.4 (C-1), 108.2 ($C(CH_3)_2$), 108.5 ($C(CH_3)_2$), 122.8 (aryl C*H*), 122.9 (aryl C*H*), 147.5 (SCH_2CN_2), 166.1 ppm (CH_2COOH); IR: $\tilde{\nu}$ = 3130 (w, cage C–H), 3035 (w, aryl C–H), 2985 (m, alkyl C–H), 2936 (m, alkyl C–H), 2567 (s, B–H), 1743 (s, C=O), 1587 (w), 1532 (w), 1384 (s), 1258 (s, C–O), 1212 (s, C–O), 1172 (m), 1101 (m), 1068 (s), 990 (m), 840 cm^{-1} (m); MS (ESI+): 784.3 $[M+H]^+$ (100%), 806.3 $[M+Na]^+$, 822.3 $[M+K]^+$; elemental analysis calcd (%) for $C_{23}H_{49}B_{18}CoN_2O_7S_2$: N 3.58, C 35.26, H 6.32; found N 3.73, C 35.03, H 6.40.

Crystal structure of 11: X-ray data were collected with an Oxford Diffraction CCD Xcalibur-S diffractometer (data reduction with CrysAlis Pro,[23] including the program SCALE3 ABSPACK[24] for empirical absorption correction) and use of $Mo_{K\alpha}$ radiation (λ = 71.073 pm) and ω-scan rotation. The structure was solved by direct methods,[25] and the refinement of all non-hydrogen atoms was performed with SHELXL97.[26] Non-hydrogen atoms were refined anisotropically. Positions of hydrogen atoms were calculated by free refinement and constrained methods by using the riding model. The structure figure (Figure 1) was drawn with the program ORTEP.[27] Crystallographic data: $C_{15}H_{39}B_{18}CoN_2O_2S_2$, M = 597.13, monoclinic space group $P2_1/n$, a = 1039.73(2), b = 2612.19(7), c = 1059.37(2) pm, β = 91.851(2)°, V = 2.8757(1) nm^3, T = −143.0(2) °C, Z = 4, 25056 reflections measured, 5863 reflections used in all calculations, R_1 [$I > 2\sigma(I)$] = 0.0327, wR_2 (all data) = 0.0712.

CCDC 834090 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre.

Peptide synthesis: Peptides were synthesized from their C-termini up to position 5 by automated solid-phase peptide synthesis (automated multiple peptide synthesis robot system from Syro, MultiSynTech, Bochum, Germany) with use of the Fmoc/*tert*-Bu strategy on a 4-(2,4-dimethoxyphenyl-Fmoc-aminomethyl)phenoxy (Rink amide) resin (30 mg with a resin loading of 0.63 mmol g^{-1}). Each coupling step was performed twice with the Fmoc-protected amino acid (10 equiv), ethyl 2-cyano-2-(hydroxyimino)acetate (Oxyma, 10 equiv), and *N*,*N*-diisopropylcarbodiimide (DIC, 10 equiv) in DMF for 40 min with amino acid and Oxyma preincubation for 2 min before addition of DIC. Removal of the Fmoc protecting group was performed with piperidine in DMF (40%, *v*/*v*) for 3 min followed by piperidine in DMF (20%, *v*/*v*) for 10 min. The last four amino acids were coupled manually. For each step, the Fmoc-protected amino acid (5 equiv) was dissolved in DMF, activated with DIC (5 equiv) and HOBt (5 equiv), and coupled to the resin overnight. Fmoc cleavage was performed twice with a solution of piperidine in DMF (20%, *v*/*v*) for 20 min. The Dde group was removed by repeated treatment with hydrazine in DMF (3%). The resin was divided equally into two. One half of the resin was treated once with a solution of **12** (3 equiv), DIC (3 equiv), and HOBt (3 equiv) overnight. The other half was initially treated with **15** (3 equiv), DIC (3 equiv), and HOBt (3 equiv) overnight; this was increased to 5 equiv each in a repeated overnight coupling step. The peptide [K^4-N_ε(**12**)]-[F^7,P^{34}]-NPY was cleaved from the resin with a mixture of TFA/thioanisole/*p*-thiocresol (90:5:5), whereas for the peptide [K^4-N_ε(**15**)]-[F^7,P^{34}]-NPY a solution of water in TFA (5%, *v*/*v*) was used. The crude peptides were purified by HPLC (Phenomenex Jupiter Proteo C_{18} column, 250 mm × 21.2 mm) with use of a gradient from 20 to 70% MeCN (0.08% TFA) in water (0.1% TFA) over 45 min at a flow rate of 10 mL min^{-1}. The peptides were analyzed by ESI mass spectrometry (Bruker Esquire HCT) and analytical HPLC (Phenomenex Jupiter Proteo C_{18} column, 4.6 × 250 mm, 5 μm) with use of a gradient from 20 to 70% MeCN (0.08% TFA) in water (0.1% TFA) over 40 min and a flow rate of 0.6 mL min^{-1}. The peptides were detected at 220 nm, and the areas of the resulting peaks were determined with the aid of EZ Chrome Elite Software (Chromtech GmbH, Idstein, Germany).

Cell culture: Cell lines were maintained under a humidified atmosphere (5% CO_2) at 37 °C and were grown to confluence before use. COS-7 cells (African green monkey, kidney) were cultured in Dulbecco's modified Eagle's medium (DMEM) containing heat-inactivated fetal calf serum (FCS, 10%), penicillin (100 U mL^{-1}), and streptomycin (100 μg mL^{-1}). HEK293 cells (human embryo kidney) stably transfected with the human Y_1 receptor and C-terminally labeled with EYFP were cultured in DMEM/Ham's F12 without L-glutamine, containing FCS (15%) and hygromycin B (100 μg mL^{-1}). HEK293 cells stably transfected with the human Y_2 receptor N-terminally labeled with an HA-tag (hemagglutinin tag) and C-terminally labeled with EYFP were maintained in DMEM/Ham's F12 without L-glutamine, containing FCS (15%) and hygromycin B (100 μg mL^{-1}).

Receptor activation studies: For signal transduction (IP accumulation) assays, COS-7 cells were seeded into 24-well plates (1×10^5 cells/well) and grown overnight. Co-transfection with hY_2R-EYFP or hY_1R-EYFP and the $G\alpha_{\Delta 6qi4myr}$ protein was performed with the aid of Metafectene. Plasmid DNA (0.32 μg) encoding the receptor and 0.08 μg encoding the chimeric G protein were used. Transfection was performed overnight at 37 °C. For labeling and stimulation, cells were marked with myo-[2-^{3}H]inositol 16 h prior to stimulation with peptide solution in concentration ranges from 10^{-5} to 10^{-12} M. Peptide stimulation was performed for 60 min. Intracellular IP levels were determined by anion-exchange chromatography, as described previously.[28] Data were analyzed with the aid of the PRISM 5.0 program (GraphPad Software). EC_{50} values were obtained from concentration/response curves. All signal transduction assays were performed in duplicate and repeated at least twice independently.

Receptor internalization: HEK293 cells stably transfected with the human Y_1 receptor C-terminally fused to EYFP (HEK293_hY1_EYFP) and HEK293 cells stably transfected with the human Y_2 receptor C-terminally fused to EYFP (HEK293_HA_hY2R_EYFP) were seeded into sterile μ-Slide 8-well plates (ibidi GmbH, Martinsried, Germany) and cultured to 80% confluency.

Prior to ligand stimulation, cells were starved in OPTI-MEM reduced-serum medium for 60 min. Cell nuclei were visualized with Hoechst 33342 nuclear stain. Stimulation was performed for 60 min with peptide solution (1 μM) in OPTI-MEM reduced-serum medium at 37 °C. Live-cell images were obtained with an Axio Observer microscope and an ApoTome imaging system (Zeiss, Jena, Germany). Fluorescence images were generated and edited with the AxioVision software Release 3.0.

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