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Preparation of 4-Halobenzoate-Containing Phosphane-Based Building Blocks for Labeling Reactions Using the Traceless Staudinger Ligation

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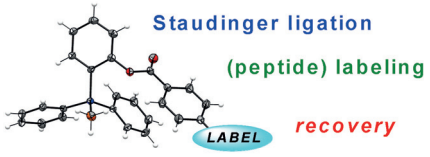
Dedicated to the memory of Dr. Rainer Evers (Universität Rostock), deceased November 2013



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Abstract Functionalized phosphane-containing key building blocks were synthesized that are suitable for the labeling of biologically active molecules by the traceless Staudinger ligation. Thus, a 2-(diphenylphosphanyl)phenyl 4-stannylbenzoate building block was converted into the 4-iodobenzoate by the introduction of iodine. The traceless Staudinger ligation was used to introduce the resulting 4-iodobenzoate moiety into selected molecules of pharmacological interest. Furthermore, the labeling procedure was used to insert the 4-iodobenzoate moiety into a peptide on solid support. Finally, a convenient recovery procedure of the key phosphane building block 2-(diphenylphosphanyl)phenol from 2-(diphenylphosphoryl)phenol was evaluated.

Key words conjugation, bioorthogonal, iodobenzoylation, recovery, labeling

Based on the pioneering work of Staudinger and Meyer,[1] two variants of the Staudinger ligation have been established.[2–4] In 2000, the so-called traceless variant was developed independently by Raines[5] and Bertozzi[6] and it is among the most powerful bioorthogonal ligation reactions.[7–9] Due to the mild reaction conditions and the formation of an amide (peptide) bond,[10] this Staudinger approach has found various applications, e.g. the labeling of bioactive molecules with fluorescence dyes[11] as well as radionuclides,[12–14] the chemoselective modification of peptides[5,15,16] and proteins,[17,18] the modification of polymers,[19] the preparation of special lactams,[20,21] or the synthesis of glycosyl amides.[22,23] The absence of cytotoxic copper salts makes the Staudinger approach interesting for potential in vitro and in vivo applications in contrast to other ligation reactions like the 1,3-dipolar Huisgen cycloaddition.[2–4] Organic azides,[24] which have no naturally available reaction partner, are easily available and are used as the starting material.[25] Further, functionalized phosphanes are easily accessible[26,27] and act as the other ligation partner.

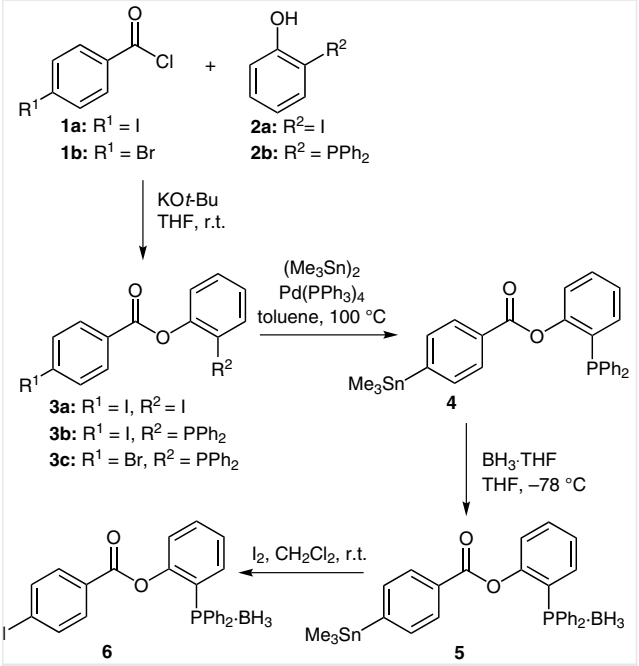
The (radio-)chemical labeling of bioactive molecules is an important tool for the study of physiological processes at the molecular level.[28,29] In particular, the introduction of organic radionuclides (e.g. ^{18}F, radioiodine, radiobromine, ^{211}At) for diagnosis [positron emission tomography (PET), single photon emission computed tomography (SPECT)] or for therapeutic approaches into high-molecular-weight compounds like peptides, proteins, oligonucleotides, or antibodies still represents a special challenge.[30–33] Normally, these molecules cannot be labeled directly due to the harsh conditions of the labeling reaction in most of the cases. To circumvent this problem, prosthetic groups, also referred to as bifunctional labeling building blocks, have been applied. For this purpose, small organic molecules that contain the desired label were synthesized capable of being linked to peptides, proteins, or antibodies under mild conditions. Such conventional radiolabeling building blocks like [^{18}F]SFB,[34,35] [$^{124/125}$I]SIB,[36,37] [^{18}F]FBAM,[38] or [^{211}At]SAB[39] are known in the literature, however, they do not exhibit bioorthogonal character and require a more complex labeling procedure and/or a protecting group strategy in most instances.

To overcome these obstacles, the traceless Staudinger approach appears to be a valuable alternative. For this purpose, an easy and convenient access to 2-phosphanylphenyl 4-halo- and 4-stannylbenzoate intermediates, which are required for the composition of building blocks for the traceless Staudinger ligation, is described herein. In this case, the introduction of electrophilic iodine to 2-(diphenylphosphanyl)phenyl 4-(trimethylstannyl)benzoate is presented. Furthermore, a solid-support-based labeling strategy for peptides is introduced that allows simple pre-purification due to the separation of the remaining starting material and by-

products. Finally, a favorable and easy recovery procedure for recovery of the starting material 2-(diphenylphosphanyl)phenol from the oxidized species 2-(diphenylphosphoryl)phenol from the ligation procedure is presented. Structures of all new compounds were confirmed by NMR and MS. Additionally, X-ray crystal structure determinations of selected key compounds were carried out to confirm their structures.

There are two common synthesis strategies for the preparation of phosphanylphenyl benzoates.[26,40] The first begins with 2-iodophenyl esters like **3a**, which is prepared from 4-iodobenzoyl chloride (**1a**) and 2-iodophenol (**2a**). In the next step, **3a** is readily reacted with diphenylphosphane under palladium catalysis. However, the regioselective introduction of the diphenylphosphanyl moiety using this reaction path fails in the case of **3a**. Alternatively, **3b** and **3c** were directly prepared from 2-(diphenylphosphanyl)phenol (**2b**) and acid chlorides **1a** and **1b**, respectively. This procedure delivered higher yields (**3b**: 91% and **3c**: 90%) in contrast to the recently used Steglich conditions.[26,40]

2-(Diphenylphosphanyl)phenyl 4-iodobenzoate (**3b**) can be employed directly for nucleophilic labeling procedures using radioiodine via isotope exchange reactions whereas the 4-bromobenzoate derivative **3c** can be used for radioiodine-for-bromine exchange.[41] Additionally, stannyl precursor **4** was prepared in a palladium-catalyzed cross-coupling from **3c** in 63% yield using hexamethyldistannane and can be utilized for electrophilic radioiodination purposes. An overview is depicted in Scheme 1.



Scheme 1 Preparation of the 4-iodobenzoyl building block **3b** and precursor **5** for the traceless Staudinger ligation

In the case of the introduction of electrophilic iodine (I^+) to **4**, sodium iodide together with reagents like Chloramine-T[42] or Iodogen[41] are applied. Iodine is introduced due to the in situ formation of iodine monochloride (ICl) followed by subsequent reaction with the stannyl precursor. In general, interhalogens like iodine monochloride as well as halogens like molecular iodine, bromine, or chlorine lead to the formation of quaternary phosphonium salts and facilitate undesired oxidation processes when treated with phosphanes.[43,44] For this reason, **4** was reacted with borane–tetrahydrofuran complex to block the phosphorus; the resulting borane-protected stannane **5** was obtained in 83% yield.

To the best of our knowledge, no direct incorporation of iodine into phosphanes has been described to date. To test **5** as starting material for these iododemetalation purposes, it was treated with a stoichiometric amount of elemental iodine in dichloromethane at ambient temperature.[45] The color of the halogen was discharged immediately and the corresponding iodo compound **6** was formed in >90% yield. Therefore, stannylated phosphane–borane adduct **5** can serve as valuable precursor for radiohalogenations. Crystals of compounds **3a** and **6** suitable for a single crystal structure determination were obtained and the molecular structures of **3a** and **6** are shown in Figure 1 and Figure 2. All bond lengths and angles are within the expected ranges. Selected crystallographic data of **3a**, **6**, **15**, and **17** are given in Table 1.

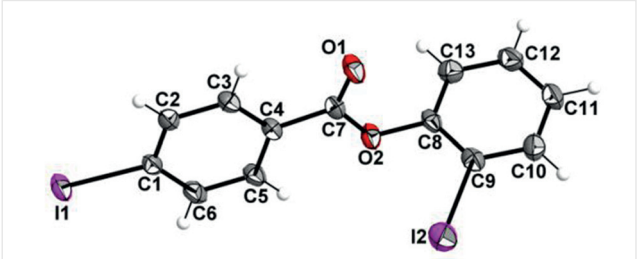


Figure 1 Molecular structure of compound **3a** (ORTEP plot, 50% probability level)

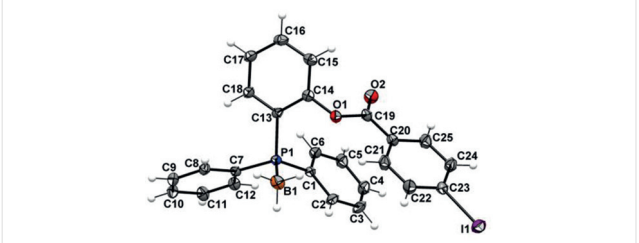
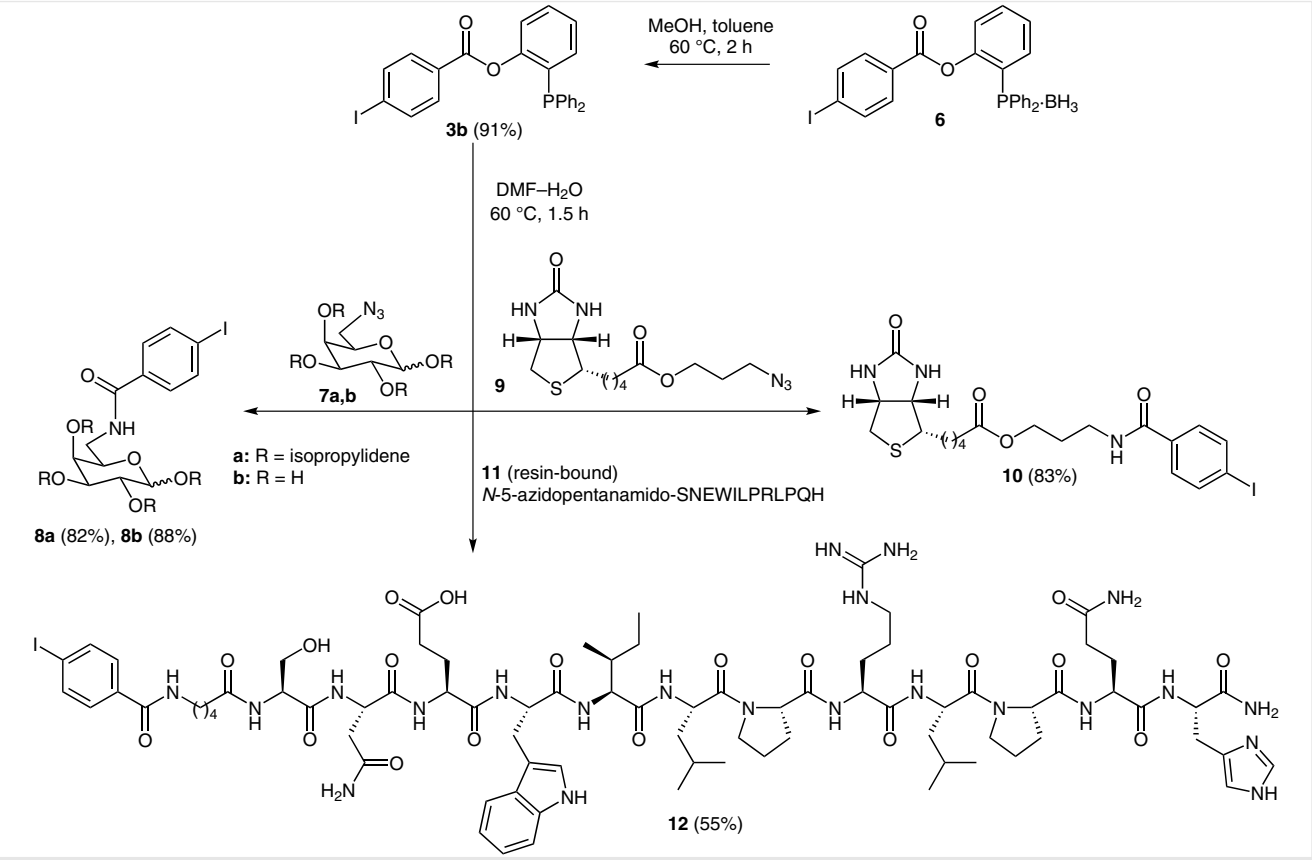


Figure 2 Molecular structure of compound **6** (ORTEP plot, 50% probability level)

Table 1 Crystallographic Data of Compounds **3a**, **6**, **15**, and **16**

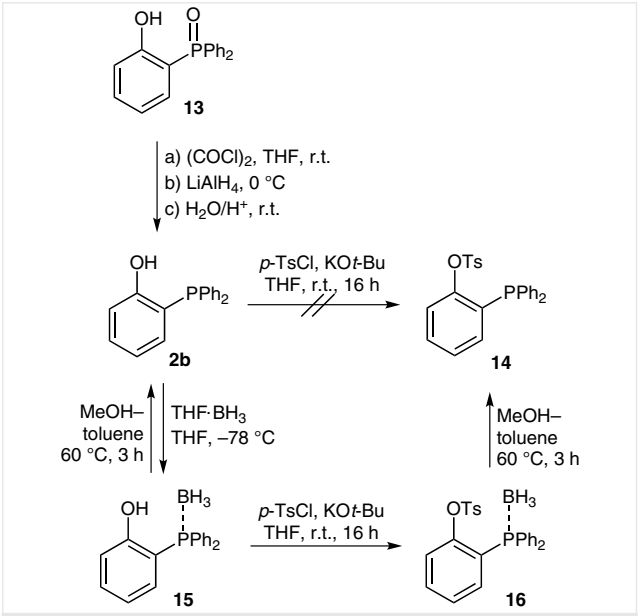
Compound	**3a**	**6**	**15**	**16**
Formula	$C_{13}H_8I_2O_2$	$C_{25}H_{21}BIO_2P$	$C_{18}H_{18}BOP$	$C_{25}H_{24}BO_3PS$
Crystal system	monoclinic	triclinic	monoclinic	triclinic
Space group	$P2_1/c$	$P\bar{1}$	$P2_1/n$	$P\bar{1}$
Unit cell dimensions (Å) or °				
a	20.527(1)	9.5804(4)	11.2305(7)	9.3578(2)
b	7.2816(4)	10.4013(4)	9.7005(6)	9.8242(2)
c	8.9829(4)	11.1366(4)	14.3539(8)	12.8433(3)
α		93.282(2)		91.837(1)
β	99.009(3)	92.793(2)	92.279(2)	103.717(1)
γ		94.960(2)		100.418(1)
*R*1, *wR*2 for $I > 2\sigma(I)$	0.051, 0.108	0.031, 0.080	0.049, 0155	0.045, 0.137

To demonstrate the efficacy of this ligation type and the margin compared to other functional groups, a selection of sample biologically active molecules were utilized for the introduction of the 4-iodobenzoate residue. The borane group of **6** is initially cleaved using a mild methanolysis reaction[40,46] to give **3b**, which was subsequently reacted with azide-modified molecules **7** and **9**. Optimal ligation conditions were found to be *N*,*N*-dimethylformamide–water (10:1), 60 °C and a reaction time of 90 minutes. The desired Staudinger products **8** and **10**, respectively, were obtained in high yields of >82%. To transfer this procedure to biomacromolecules,[6,15,16,46–50] the pharmacologically interesting peptide with the SNEW[51] amino acid sequence was chosen and modified with a 5-azidopentanamido moiety connected to the N-terminus. Peptide **11** was achieved via solid phase peptide synthesis (SPPS)[50] by the introduction of 5-azidopentanoic acid according to a recently published procedure.[35,52] Next, the traceless Staudinger ligation was executed on solid support to introduce the 4-iodobenzoate label. Thus, peptide **11** bound on resin was reacted with phosphane **3b** to yield **12** under the described reaction conditions. In the next step, peptide **12** still bound on resin was washed to remove remaining starting material and byproducts such as the oxidized phosphane species. Finally, the desired peptide **12** was obtained after deprotection and cleavage from the resin in 55% yield after HPLC purification (Scheme 2).



Scheme 2 Iodobenzoylation of selected biologically active molecules using the traceless Staudinger ligation

Key intermediate **2b** acts as starting material for all building blocks discussed in this paper and was usually prepared from 2-iodophenol (**2a**) and diphenylphosphane in a palladium-catalyzed P–C cross coupling reaction.[26,53] A small amount of oxidized species **13** was obtained (≤1%) in addition to the desired product in every preparation of **2b**. Furthermore, compound **13** was generally obtained as by-product in all traceless Staudinger ligations. For this purpose, a strategy for the recirculation of **2b** from **13** was evaluated. Horner and co-workers described a procedure for the reduction of phosphane oxides with lithium aluminum hydride[54] under harsh reaction conditions. Other groups reported electroreduction[55] or the use of special reductants.[56] An alternative two-step one-pot procedure published by the group of Gilheany employs a mild conversion of phosphane oxides into the desired phosphanes using oxalyl chloride to generate the chlorophosphonium salt followed by reduction with lithium aluminum hydride.[57,58] In our case, it was mandatory to use two equivalents of oxalyl chloride and lithium aluminum hydride. After careful quenching with water, it is necessary to acidify the resulting solution to neutral pH. Using this protocol, compound **2b** was successfully obtained from **13** in >90% yield (Scheme 3).



Scheme 3 Recovery of phosphane **2b** and tosylation procedure

Finally, the tosylation of OH groups present in phosphanes of oxidation state +3 remains an important item, especially for the introduction of sulfonates that serve as good leaving groups for nucleophilic substitutions. The direct tosylation of OH groups of unprotected phosphanes like **2b** with 4-toluenesulfonyl chloride to yield **14** is not possible.[13] Alternatively, it was possible to carry out the introduction using the respective halogen derivative which was treated with silver tosylate,[12] but this is only possible using aliphatic halogens.

For aromatic compounds like **2b**, the phosphorus has to be blocked. Thereafter, tosylation was successful with borane adduct **15**, which was reacted with 4-toluenesulfonyl chloride in the presence of potassium *tert*-butoxide as base in tetrahydrofuran at ambient temperature to obtain compound **16** in 69% yield. Finally, **16** was dissolved in a methanol–toluene mixture (2:1) and maintained at 60 °C for three hours to deprotect the phosphanyl group to obtain the desired product **14** in 90% yield (Scheme 3). Crystals were obtained successfully from compounds **15** and **16** suitable for a single crystal structure determination. Both molecular structures are presented in Figure 3 and Figure 4. In crystals of **15** the molecules adapt two different orientations. This is treated in the structure refinement by a split model with the OH group being attached to C2 for 81% of the molecules (C2A, O1A, C8A), and for the remaining amount of 19% to C8 (C8B O1B, C2B). As pointed out for **3a** and **6**, all bond distances and angles are found within the expected ranges.

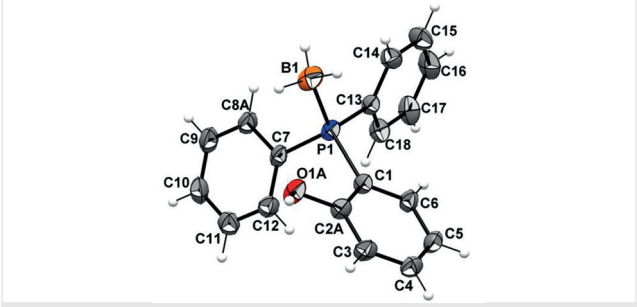


Figure 3 Molecular structure of compound **15** (ORTEP plot, 50% probability level)

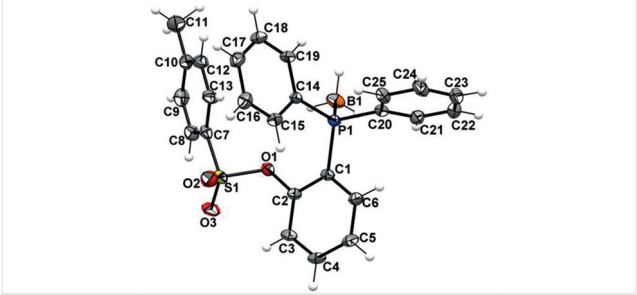


Figure 4 Molecular structure of compound **16** (ORTEP plot, 50% probability level)

In conclusion, we have reported a straightforward synthesis protocol for the preparation of aromatic halobenzoate phosphane building blocks suitable for labeling purposes using the traceless Staudinger ligation. The introduction of electrophilic iodine into borane-protected phosphanes has been executed successfully. The introduction of this 4-iodobenzoate moiety into a pharmacologically relevant azide-containing peptide on solid phase is demonstrated.

Furthermore, a convenient procedure for the recovery of the phosphane key building block and the successful tosylation of this molecule is presented.

NMR spectra were recorded on a Varian Inova-400 for ^{1}H, ^{13}C, and ^{31}P spectra using the solvent shifts for ^{1}H and ^{13}C and H_3PO_4 for ^{31}P spectra as the internal standard. All melting points were determined on a Galen III (Cambridge Instruments) melting point apparatus (Leica, Vienna, Austria) and are uncorrected. Elemental analyses (C, H, N, S) were conducted using the Hekatech CHNS elemental analyser EuroEA 3000, their results were found to be in good agreement (± 0.3%) with the calculated values. Anhyd solvents (*N*,*N*-dimethylacetamide, THF, toluene) were purchased from Sigma Aldrich (anhyd, over molecular sieves, 99.7%) and other chemicals used for the syntheses were purchased from commercial suppliers and were used as received. Chromatographic separations and TLC detections were carried out with Merck Silica Gel 60 (63–200 µm) and Merck Silica Gel 60 F_{254} sheets, respectively. TLCs were developed by visualization under UV light (λ = 254 nm). All reactions concerning the palladium-catalyzed coupling reactions, the reduction procedure with $NaBH_4$ and the borane–phosphane adduct formation were carried out under an argon atmosphere using Schlenk techniques. Diffraction data were collected with a Bruker-Nonius Apex-X8 CCD- (**3a**) or a Bruker-Nonius-Apex-II- (**6**, **15**, **16**) diffractometer using graphite-monochromated Mo-Kα radiation (λ = 0.71073 Å). All diffraction measurement was done at –100 °C. The unit cell dimensions was recorded and refined using the angular settings of up to 10000 reflections and the structures was solved by direct methods and refined against F^2 on all data by full-matrix least-squares using the program suits from Sheldrick.[59,60] All non-hydrogen atoms were refined anisotropically; all hydrogen atoms bonded to C or B atoms were placed on geometrically calculated positions and refined using a riding model. CCDC 1007868 (**3a**), CCDC 1009051 (**6**), CCDC 1009306 (**15**), and CCDC 1009398 (**16**) contain the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Phenyl Benzoates 3a–c; General Procedure

KO*t*-Bu (1 equiv) was added to a solution of phenol **2a** or **2b** (1 equiv) in anhyd THF at r.t. The respective benzoyl chloride **1a** or **1b** (1.2 equiv) was added and the solution was allowed to stir overnight. Sat. $NaHCO_3$ (10 mL) was added and the organic layer separated. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed. The crude product was purified by column chromatography (PE–EtOAc, 20:1).

2-Iodophenyl 4-Iodobenzoate (3a)

Following the general procedure, KO*t*-Bu (153 mg, 1.36 mmol), 2-iodophenol (**2a**, 300 mg, 1.36 mmol), and 4-iodobenzoyl chloride (**1a**, 436 mg, 1.63 mmol) in anhyd THF (6 mL) yielded **3a** (593 mg, 95%) as a colorless solid. NMR and MS spectra were found to be identical with those in the literature.[25]

2-(Diphenylphosphanyl)phenyl 4-Iodobenzoate (3b)

Following the general procedure, KO*t*-Bu (101 mg, 0.90 mmol), 2-(diphenylphosphanyl)phenol (**2b**, 250 mg, 0.90 mmol), and 4-iodobenzoyl chloride (**1a**, 287 mg, 1.08 mmol) in anhyd THF (6 mL) yielded **3b** (415 mg, 91%) as a colorless solid. NMR and MS spectra were found to be identical with those in the literature.[25]

2-(Diphenylphosphanyl)phenyl 4-Bromobenzoate (3c)

Following the general procedure, KO*t*-Bu (120 mg, 1.08 mmol), 2-(diphenylphosphanyl)phenol (**2b**, 300 mg, 1.08 mmol), and 4-bromobenzoyl chloride (**1b**, 284 mg, 1.29 mmol) in anhyd THF (6 mL) yielded **3c** (450 mg, 90%) as a colorless solid; mp 142 °C; R_f = 0.47 (PE–EtOAc, 8:1).

^{1}H NMR (400 MHz, C_6D_6): δ = 6.83 (t, ^{3}J = 7.4 Hz, 1 H, H4), 6.98–7.12 (m, 10 H, H5, H6, *m*-H, *m*-H′, *p*-H), 7.24 (dd, ^{3}J = 8.0 Hz, $^{4}J_{H,P}$ = 4.1 Hz, 1 H, H3), 7.32–7.40 (m, 4 H, *o*-H), 7.68 (d, ^{3}J = 8.2 Hz, 2 H, *o*-H′).

^{13}C NMR (101 MHz, C_6D_6): δ = 123.1 (C6), 126.5 (C4), 128.6, 128.7 (*i*-C′, *p*-C′), 128.9 (d, $^{3}J_{C,P}$ = 7.4 Hz, *m*-C), 129.2 (*p*-C), 130.1 (C5), 131.6 (d, $^{1}J_{C,P}$ = 15.5 Hz, C2), 131.8, 131.9 (*m*-C′, *p*-C′), 134.0 (C3), 134.4 (d, $^{2}J_{C,P}$ = 21.0 Hz, *o*-C), 136.2 (d, $^{1}J_{C,P}$ = 11.0 Hz, *i*-C), 153.5 (d, $^{1}J_{C,P}$ = 18.0 Hz, C1), 163.5 (C=O).

^{31}P NMR (162 MHz, C_6D_6): δ = –14.5.

MS (ESI+): *m*/*z* = 463 (M^+ + H, ^{81}Br), 461 (M^+ + H, ^{79}Br).

Anal. Calcd for $C_{25}H_{18}BrO_2P$: C, 65.09; H, 3.93. Found: C, 65.01; H, 3.90.

2-(Diphenylphosphanyl)phenyl 4-(Trimethylstannyl)benzoate (4)

Under an argon atmosphere, compound **3c** (155 mg, 0.34 mmol) and hexamethyldistannane (143 mg, 0.44 mmol) were dissolved in anhyd toluene (5 mL), $Pd(PPh_3)_4$ was added in catalytic amounts and the resulting mixture was heated at 100 °C overnight. After cooling to r.t., H_2O (10 mL) was added and the organic layer was separated. The aqueous layer was extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed. The crude product was purified by column chromatography (PE–EtOAc, 20:1) to give **4** (116 mg, 63%) as a yellowish syrup; R_f = 0.66 (PE–EtOAc, 5:1).

^{1}H NMR (400 MHz, C_6D_6): δ = 0.11 (s, 9 H, $SnMe_3$), 6.85 (t, ^{3}J = 7.5 Hz, 1 H, H4), 6.99–7.09 (m, 8 H, H5, H6, *m*-H, *p*-H), 7.27 (dd, ^{3}J = 7.9 Hz, $^{4}J_{H,P}$ = 4.1 Hz, 1 H, H3), 7.34 (d, ^{3}J = 7.9 Hz, 2 H, *m*-H′), 7.36–7.42 (m, 4 H, *o*-H), 8.14 (d, ^{3}J = 7.9 Hz, 2 H, *o*-H′).

^{13}C NMR (101 MHz, C_6D_6): δ = –10.2 ($SnMe_3$), 123.3 (C6), 126.4 (C4), 128.9 (d, $^{3}J_{C,P}$ = 6.9 Hz, *m*-C), 129.1 (*p*-C), 129.6 (*o*-C′), 129.9 (*i*-C′), 130.2 (C5), 131.4 (d, $^{1}J_{C,P}$ = 15.9 Hz, C2), 134.1 (C3), 134.4 (d, $^{2}J_{C,P}$ = 20.6 Hz, *o*-C), 136.0 (*m*-C′), 136.2 (d, $^{1}J_{C,P}$ = 11.0 Hz, *i*-C), 150.0 (*p*-C′), 154.1 (d, $^{1}J_{C,P}$ = 18.0 Hz, C1), 164.8 (C=O).

^{31}P NMR (162 MHz, C_6D_6): δ = –16.2.

MS (ESI+): *m*/*z* = 569 (M^+ + Na), 547 (M^+ + H).

Anal. Calcd for $C_{28}H_{27}O_2PSn$: C, 61.68; H, 4.99. Found: C, 61.55; H, 4.89.

2-(Diphenylphosphanyl)phenyl 4-(Trimethylstannyl)benzoate–Borane Adduct (5)

Under an argon atmosphere, phosphane **4** (85 mg, 0.16 mmol) was dissolved in anhyd THF (4 mL), the solution was cooled to –78 °C and 1 M BH_3·THF (0.16 mL, 0.16 mmol) was added slowly. The temperature of the mixture was allowed to rise to r.t. over 2 h. The solvent was removed under reduced pressure and the crude product was purified by column chromatography (PE–EtOAc, 20:1) to yield **5** (72 mg, 83%) as a colorless solid; mp 120 °C; R_f = 0.40 (PE–EtOAc, 5:1).

^{1}H NMR (400 MHz, C_6D_6): δ = 0.13 (s, 9 H, $SnMe_3$), 1.17–1.47 (m, 3 H, BH_3), 6.75 (t, ^{3}J = 7.6 Hz, 1 H, H4), 6.88–6.97 (m, 6 H, *m*-H, *p*-H), 7.04 (t, ^{3}J = 7.8 Hz, 1 H, H5), 7.17 (d, 1 H, H6), 7.28–7.38 (m, 3 H, *m*-H′, H3), 7.71–7.81 (m, 4 H, *o*-H), 7.98 (d, ^{3}J = 7.9 Hz, 2 H, *o*-H′).

^{11}B NMR (128 MHz, C_6D_6): δ = –36.3.

^{13}C NMR (101 MHz, C_6D_6): δ = –9.8 ($SnMe_3$), 124.8 (d, $^3J_{C,P}$ = 5.2 Hz, C6), 125.9 (d, $^3J_{C,P}$ = 8.6 Hz, C4), 128.9 (d, $^3J_{C,P}$ = 10.3 Hz, *m*-C), 129.1 (d, $^2J_{C,P}$ = 7.4 Hz, C3), 129.6 (*i*-C′), 129.7 (*o*-C′), 131.1 (d, $^4J_{C,P}$ = 1.9 Hz, *p*-C), 132.6 (d, $^4J_{C,P}$ = 1.3 Hz, C5), 133.6 (d, $^2J_{C,P}$ = 9.7 Hz, *o*-C), 134.9 (d, $^1J_{C,P}$ = 7.7 Hz, *i*-C), 135.9 (*m*-C′), 150.3 (*p*-C′), 153.5 (d, $^1J_{C,P}$ = 2.9 Hz, C1), 164.2 (C=O).

^{31}P NMR (162 MHz, C_6D_6): δ = 19.9.

MS (ESI+): *m*/*z* = 583 (M^+ + Na), 569 (M^+ + Na – BH_3).

Anal. Calcd for $C_{28}H_{30}BO_2PSn$: C, 60.16; H, 5.41. Found: C, 60.25; H, 5.31.

2-(Diphenylphosphanyl)phenyl 4-Iodobenzoate–Borane Adduct (6)

Phosphane–borane adduct **5** (100 mg, 0.18 mmol) was dissolved in CH_2Cl_2 (4 mL) and I_2 (45 mg, 0.18 mmol) was added and the resulting mixture was stirred at r.t. for 30 min. The solvent was removed and the crude product was purified by column chromatography (PE–EtOAc, 20:1) to yield **6** (89 mg, 95%) as a colorless solid. NMR and MS spectra were found to be identical to those described in the literature.[25]

6-Deoxy-1,2:3,4-di-*O*-isopropylidene-6-(4-iodobenzamido)-α-D-galactopyranose (8a)

Azido compound **7a** (67 mg, 0.23 mmol) and phosphane **3b** (100 mg, 0.20 mmol) were dissolved in a mixture of DMF (3 mL) and H_2O (0.3 mL) and heated at 60 °C for 2 h. The solvent was removed and the residue was purified by column chromatography (PE–EtOAc, 4:1 → 2:1) to give **8a** (79 mg, 82%) as a colorless solid; mp 177 °C; R_f = 0.27 (PE–EtOAc, 2:1).

^{1}H NMR (400 MHz, C_6D_6): δ = 1.01 (s, 3 H, CH_3), 1.12 (s, 3 H, CH_3), 1.39 (s, 3 H, CH_3), 1.46 (s, 3 H, CH_3), 3.40–3.49 (m, 1 H, H6a), 3.83 (dd, $^3J_{4,5}$ = 1.5 Hz, $^3J_{3,4}$ = 8.0 Hz, 1 H, H4), 4.00–4.08 (m, 1 H, H6b), 4.14 (dd, ^4J = 2.5 Hz, ^3J = 5.0 Hz, 1 H, H2), 4.22 (dd, ^4J = 1.5 Hz, ^3J = 9.0 Hz, 1 H, H5), 4.41 (dd, $^3J_{2,3}$ = 2.5 Hz, $^3J_{3,4}$ = 8.0 Hz, 1 H, H3), 5.43 (d, ^3J = 5.0 Hz, 1 H, H1), 6.30 (br s, 1 H, NH), 7.21 (d, ^3J = 8.4 Hz, 2 H, *m*-H), 7.34 (d, ^3J = 8.4 Hz, 2 H, *o*-H).

^{13}C NMR (101 MHz, C_6D_6): δ = 24.3, 25.0, 26.2, 26.3 (4 CH_3), 41.2 (C6), 66.8 (C5), 71.2 (C2), 71.4 (C3), 72.0 (C4), 96.8 (C1), 98.1 (*p*-C), 108.8, 109.3 (2 C_q), 132.2 (*o*-C), 134.5 (*i*-C), 137.8 (*m*-C), 166.5 (C=O).

MS (ESI+): *m*/*z* = 512 (M^+ + Na), 490 (M^+ + H).

Anal. Calcd for $C_{19}H_{24}INO_6$: C, 46.64; H, 4.94; N, 2.86. Found: C, 46.66; H, 4.92; N, 2.90.

6-Deoxy-6-(4-iodobenzamido)-D-galactose (8b)

Azido compound **7b** (95 mg, 0.46 mmol) and phosphane **3b** (259 mg, 0.51 mmol) were dissolved in a mixture of DMF (5 mL) and H_2O (0.5 mL) and heated at 60 °C for 2 h. The solvent was removed and the residue was purified by column chromatography ($CHCl_3$–MeOH, 10:1) to give **8b** (166 mg, 88%) as a colorless syrup; R_f = 0.34 ($CHCl_3$–MeOH, 5:1).

^{1}H NMR (400 MHz, CD_3OD): δ = 3.44–3.54 (m, 3 H, H_{Gal}), 3.67–3.80 (m, 3 H, H_{Gal}), 4.42 (d, $^3J_{1,2}$ = 3.8 Hz, 1 H, H1), 7.58 (d, ^3J = 8.6 Hz, 2 H, *m*-H), 7.84 (d, ^3J = 8.6 Hz, 2 H, *o*-H).

^{13}C NMR (101 MHz, CD_3OD): δ = 42.0 (C6), 70.9, 73.7, 74.3, 74.9 (C2 to C5), 98.8 (C1), 101.0 (*p*-C), 130.1 (*o*-C), 132.2 (*i*-C), 139.0 (*m*-C), 169.7 (C=O).

MS (ESI+): *m*/*z* = 432 (M^+ + Na), 410 (M^+ + H).

Anal. Calcd for $C_{13}H_{16}INO_6$: C, 38.16; H, 3.94; N, 3.42. Found: C, 38.22; H, 3.88; N, 3.55.

3-(4-Iodobenzamido)propyl 5-[(3a*S*,4*S*,6a*R*)-2-Oxohexahydro-1*H*-thieno[3,4-*d*]imidazol-4-yl]pentanoate (10)

Azido compound **9** (66 mg, 0.20 mmol) and phosphane **3b** (113 mg, 0.22 mmol) were dissolved in a mixture of DMF (3 mL) and H_2O (0.3 mL) and heated at 60 °C for 2 h. The solvent was removed and the residue was purified by column chromatography (EtOAc → EtOAc–EtOH, 2:1) to give **10** (89 mg, 83%) as a colorless syrup; R_f = 0.24 ($CHCl_3$–MeOH, 10:1).

^{1}H NMR (400 MHz, CD_3OD): δ = 1.38–1.78 (m, 5 H, biotin), 1.90–2.03 (m, 2 H, CH_2), 2.34 (t, ^3J = 7.3 Hz, 2 H, CH_2CO), 2.70 (d, *J* = 12.8 Hz, 1 H, biotin), 2.85–3.01 (m, 2 H, biotin), 3.16–3.22 (m, 1 H, biotin), 3.46 (t, ^3J = 6.7 Hz, 2 H, CH_2N), 4.17 (t, ^3J = 6.2 Hz, 2 H, CH_2O), 4.29 (dd, *J* = 4.4 Hz, ^3J = 7.8 Hz, 1 H, biotin), 4.48 (dd, *J* = 4.7 Hz, ^3J = 7.5 Hz, 1 H, biotin), 7.57 (d, ^3J = 8.4 Hz, 2 H, *m*-H), 7.84 (d, ^3J = 8.4 Hz, 2 H, *o*-H).

^{13}C NMR (101 MHz, CD_3OD): δ = 26.0, 29.5, 29.6, 29.7 (3 CH_2), 34.9 (NCH_2), 38.1 (CH_2CO), 41.1 (SCH_2), 57.0 (SCH), 61.7 (NCH), 63.3 (OCH_2), 63.4 (CHN), 99.2 (*p*-C), 130.0 (*o*-C), 135.3 (*i*-C), 139.0 (*m*-C), 166.1, 169.4, 175.4 (3 C=O).

MS (ESI+): *m*/*z* = 554 (M^+ + Na), 532 (M^+ + H).

Anal. Calcd for $C_{20}H_{26}IN_3O_4S$: C, 45.20; H, 4.93; N, 7.91. Found: C, 45.22; H, 4.90; N, 8.01.

N-[5-(4-Iodobenzamido)pentanamido]-SNEWILPRLPQH (12)

Resin-bound peptide **11**[33] (55 mg) was swollen in DMF (2 mL) for 20 min. Phosphane **3b** (10 mg) was dissolved in a mixture of DMF (1 mL) and H_2O (0.1 mL) and incubated at 60 °C for 6 h. After cooling to r.t., the resin was washed with DMF and CH_2Cl_2. The resulting peptide was deprotected and cleaved from the solid support with TFA–thioanisole–H_2O–triisopropylsilane (94:2.5:2.5:1) at r.t. for 4 h. The solution containing the crude peptide was concentrated in a stream of N_2 and precipitated by addition of cold Et_2O, and the solid was collected by filtration. Peptide **12** was obtained as a colorless powder after purification by preparative HPLC (Agilent Technologies 1200 system equipped with automatic injector and a UV detector, column: Macherey-Nagel EC 250 × 4.6 Nucleosil Standard 100-7 C18, 25–40% MeCN + 0.1% TFA over 20 min, t_R = 21.2 min) and lyophilization, yield: 55%.

MS (ESI+): *m*/*z* = 1698 (M^+ + H), 850 (M^{2+} + 2 H), 566 (M^+ – iodobenzamidyl).

2-(Diphenylphosphanyl)phenol (2b); Recovery Procedure

Compound **13** (100 mg, 0.34 mmol) was dissolved in anhyd THF (6 mL) under argon. Oxalyl chloride (108 mg, 0.85 mmol) was added dropwise and the mixture was stirred for 30 min. 1 M $LiAlH_4$ (0.85 mL, 0.85 mmol) was added at 0 °C and the mixture was warmed to r.t. over 30 min. The reaction was then quenched by adding 1 M HCl (10 mL) and extracted with EtOAc (3 × 10 mL). Then, the organic layer was separated, dried (Na_2SO_4), the solvent was removed and the crude product was purified by column chromatography (PE–EtOAc, 10:1) to give **2b** (212 mg, 69%) as colorless crystals.

IR (ATR): 3226 (OH), 1577, 1433, 1284, 746, 696 cm^{-1}.

NMR and MS spectra were identical with those described in the literature.[25]

2-(Diphenylphosphanyl)phenyl *p*-Tosylate–Borane Adduct (16)

Compound **15** (200 mg, 0.68 mmol) and KO*t*-Bu (100 mg, 0.89 mmol) were dissolved in anhyd THF (6 mL) and *p*-TsCl (170 mg, 0.89 mmol) was added and the resulting mixture was stirred at r.t. overnight. Sat. $NaHCO_3$ was added and extracted with EtOAc (3 × 10 mL). The combined organic layers were dried (Na_2SO_4), the solvent was removed, and the crude product was purified by column chromatography (PE–EtOAc, 4:1) to give **16** (212 mg, 69%) as colorless crystals; mp 188 °C; R_f = 0.40 (PE–EtOAc, 3:1).

IR (KBr): 3075, 2925: 2416 (BH_3, as), 2380 (BH_3, s), 1435, 1355 (S=O, as), 1200 (S=O), 1179, 1071 cm^{-1}.

^1H NMR (400 MHz, C_6D_6): δ = 1.58–2.48 (br m, 3 H, BH_3), 1.76 (s, 3 H, CH_3), 6.54 (d, ^3J = 8.3 Hz, 2 H, *m*-H_{Ts}), 6.65 (t, ^3J = 7.6 Hz, 1 H, H5), 6.87–7.03 (m, 6 H, *m*-H, *p*-H), 7.34 (d, ^3J = 8.3 Hz, 2 H, *o*-H_{Ts}), 7.59–7.67 (m, 6 H, *o*-H, H4, H6), 7.90 (dd, ^3J = 3.5 Hz, ^3J = 8.3 Hz, 1 H, H3).

^{13}C NMR (101 MHz, C_6D_6): δ = 21.2 (CH_3), 119.4 (d, ^3J = 4.2 Hz, C6), 120.7 (d, ^1J = 51.9 Hz, C2), 125.7 (d, ^3J = 9.9 Hz, C4), 128.5 (*o*-C_{Ts}), 128.8 (d, ^3J = 10.3 Hz, *m*-C), 129.2 (d, ^1J = 58.3 Hz, *i*-C), 129.5 (*m*-C_{Ts}), 131.0 (d, ^4J = 2.4 Hz, *p*-C), 133.4 (d, ^2J = 9.9 Hz, *o*-C), 133.6 (d, ^2J = 2.0 Hz, C5), 133.7 (*i*-C_{Ts}), 136.7 (d, ^4J = 10.1 Hz, C3), 145.0 (*p*-C_{Ts}), 153.1 (C1).

^{31}P NMR (162 MHz, C_6D_6): δ = 21.8 (br m).

MS (ESI+): *m*/*z* = 448 (M^+ + 2 H), 433 (M^+ – BH_3).

Anal. Calcd for $C_{25}H_{24}BO_3PS$: C, 67.28; H, 5.42. Found: C, 67.22; H, 5.39.

2-(Diphenylphosphanyl)phenyl *p*-Tosylate (14)

Compound **16** was dissolved in a mixture of toluene (4 mL) and MeOH (2 mL) and the resulting solution was heated at 60 °C for 3 h (TLC monitoring). The solvent was removed and the residue was purified by column chromatography (PE–EtOAc, 4:1) to give **14** (212 mg, 69%) as colorless crystals; mp 98 °C; R_f = 0.58 (PE–EtOAc, 3:1).

IR (KBr): 3058. 2924, 1433, 1358 (S=O, as), 1196 (S=O, s), 852, 725 cm^{-1}.

^1H NMR (400 MHz, C_6D_6): δ = 1.78 (s, 3 H, CH_3), 6.60 (d, ^3J = 8.3 Hz, 2 H, *m*-H_{Ts}), 6.70 (t, ^3J = 7.5 Hz, 1 H, H5), 6.87–6.92 (m, 2 H, H4, H6), 6.97–7.02 (m, 6 H, *m*-H, *p*-H), 7.17–7.23 (m, 4 H, *o*-H), 7.67 (dd, ^3J = 4.4 Hz, ^3J = 8.9 Hz, 1 H, H3), 7.79 (d, ^3J = 8.3 Hz, 2 H, *o*-H_{Ts}).

^{13}C NMR (101 MHz, C_6D_6): δ = 21.2 (CH_3), 122.3 (C6), 127.0 (C4), 128.8 (d, ^3J = 7.1 Hz, *m*-C), 129.0 (*o*-C_{Ts}), 129.1 (d, ^4J = 2.0 Hz, *p*-C), 129.5 (*m*-C_{Ts}), 130.7 (C5), 131.9 (d, ^2J = 19.3 Hz, C3), 134.1 (d, ^2J = 20.5 Hz, *o*-C), 144.7 (*p*-C_{Ts}), 153.3 (d, ^2J = 19.0 Hz, C1).

^{31}P NMR (162 MHz, C_6D_6): δ = –16.5.

MS (ESI+): *m*/*z* = 433 (M^+ + H).

Anal. Calcd for $C_{25}H_{21}O_3PS$: C, 69.43; H, 4.89. Found: C, 69.48; H, 4.93.

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Supporting Information

Supporting information for this article, including ^1H and ^{13}C NMR spectra, for this article is available online at http://dx.doi.org/10.1055/s-0034-1379487.

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